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LUTHER PARRIS BYARS, 1887-1950

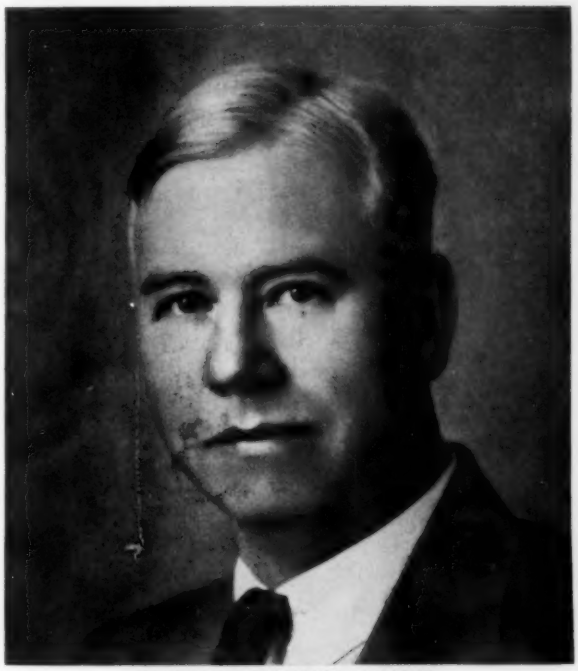
G. W. Keitt

Luther Parris Byars, a life member of the American Phytopathological Society, died suddenly at his summer home at Murrell's Inlet, South Carolina, June 4, 1950.

The son of Edward Harrison and Nancy Parris Byars, Luther was born at Forest City, North Carolina, September 25, 1887. When he was 3 years old his family moved to Marion, South Carolina, where he received his early education. He graduated from Clemson College in 1909 with the B.S. degree. In 1909-10 he engaged in commercial work on cotton, and 1910-11 was assistant in plant pathology at the South Carolina Agricultural Experiment Station. The period 1911-14 was spent in graduate work at the University of Wisconsin, from which he was granted the M.S. degree in 1913 and the Ph.D. in 1919, majoring in plant pathology. He served as assistant in botany at the University of Wisconsin for 3 semesters, and in the summers of 1912 and 1913 was a special agent in the Bureau of Plant Industry, United States Department of Agriculture. In 1913-14 he was a scholar in plant pathology at Wisconsin. On July 1, 1914 he accepted an appointment as Pathologist in the Offices of Nematology and Cotton and Truck Crop Diseases of the Bureau of Plant Industry, United States Department of Agriculture, in which he served until March 18, 1920.

In his work in the United States Department of Agriculture he continued and expanded researches already well advanced at Wisconsin on plant diseases incited by nematodes, giving special attention to these diseases on small grains, sugar beets, and potatoes. An important early contribution was the development of methods for culture and study of nematodes *in vitro*. His contributions to the knowledge of diseases incited by nematodes were reported in his doctoral dissertation and in a series of publications, of which 16 are available to the present writer. He was a Fellow of the American Association for the Advancement of Science and a member, besides the American Phytopathological Society, of the Botanical Society of America, the Botanical Society of Washington, and Sigma Xi.

Luther Byars had a rare gift for friendship and a deep feeling of love and loyalty for his home community and state. While visiting Marion soon after the First World War, he was urged by an elderly friend to return home and take over the local Ford automobile agency. After careful deliberation, he decided to accept this proposal, although reluctant to give up a profession in which he had already made valuable contributions and developed many friendships and associations. This was the beginning of a new and very happy and successful phase of his life. His industry, fine character and judgment, high ideals of friendship and service, and a high order of business ability enabled him to become one of the beloved and respected leaders in his community and state. His business prospered and expanded to include the presidency of the Citizens' Bank of Marion and ownership of a large plantation. He held many posts of trust and public service. He was a member of the Boards of Directors of the Marion Federal Savings and Loan Association and of the Palmetto State Life Insurance Company. For 2 terms, he was a representative of Marion County in the State Legislature. He was a member of the State Board of Public Welfare and served on the Boards of Trustees of Coker College and Limestone College. He was active in church and civic affairs and enjoyed a host of friendships. He loved the out of doors and was an enthusiastic hunter and fisherman.



LUTHER PARRIS BYARS
1887—1950

In July, 1922, he married Miss Lurline Margaret Patton of Jackson, Mississippi, who made a charming and hospitable home and shared fully in his life work and interests. Their oldest child, Luther Patton Byars, gave his life for his country in Europe on D Day in the Second World War. Surviving, with their mother, are one daughter, Mrs. John Stedman, and a son, John Arthur Byars, of Marion.

Luther Byars lived a full life, rich in service and friendships. In his passing the American Phytopathological Society has lost a valued member, and his community and state have lost a beloved and distinguished citizen.

The titles of several of Dr. Byars' publications follow:

1920. Soil disinfection with hot water to control the root-knot nematode and parasitic soil fungi.

U. S. Dept. Agr. Bul. 818. (With W. W. Gilbert)
1920. The nematode disease of wheat caused by *Tylenchus tritici*. U. S. Dept. Agr. Bul. 842.
1920. A nematode disease of red clover and strawberry in the Pacific Northwest. Phytopathology 10: 91-95.
1921. Notes on the citrus-root nematode, *Tylenchulus semipenetrans* Cobb. Phytopathology 11: 90-94.

E. W. BODINE, 1906-1952

L. W. Durrell

E. W. BODINE
1906-1952

Dr. E. W. Bodine, Plant Pathologist in Charge of Western Division of Shell Chemical Corporation, died of a heart attack August 11, 1952, at the age of 46. At the time of his death he was leaving for Denver, Colorado, to establish a new product development with the Corporation's Julius Hyman Division.

Dr. Bodine was born in Denver, Colorado, January 5, 1906. He received his B.S. degree from Colorado A & M College in 1928 and his M.S. in 1929. In 1944 he received his Ph.D. degree from University of California. From 1930 to 1934 Dr. Bodine was Assistant Professor of Botany and Plant Pathology at Colorado A & M College, Fort Collins, Colorado; Associate Professor 1935-40, and Professor 1940-42. On leave from 1942-44 he was Plant Pathologist on a disease survey in the mountain states for U. S. Department of Agriculture. In 1945 he accepted a position with the Shell Oil Company and assisted in establishing the research laboratory of that Company at Modesto, California.

Dr. Bodine was a member of Colorado-Wyoming Academy of Science, and of the American Phytopathological Society. His research over the years covered work on wheat smut, onion diseases, pepper blight, peach mosaic, ringspot of apricot, golden net disease of peach, X-disease of peach, and rasp leaf of cherry.

He was a good teacher, enthusiastic and greatly respected by his students and his associates. His friendliness and cooperative spirit were distinctive; he could start work on a pathology problem and inspire confidence in a group of growers and make them his friends. His greatest interest, however, was in plant pathological research. In his chosen field he was a hard and indefatigable worker, a master technician, with an especial genius for solving pathological problems.

The following are some of Dr. Bodine's more important publications:

1931. Double plate method used for culturing Tilletia levis. Science 74: 341.
1935. Sclerotinia wilt of Canada thistle. Phytopathology 25: 963-964.
1935. Blight of peppers. Colorado Agr. Exp. Sta. Bull. 85.
1936. Peach mosaic disease in Colorado. Colorado Agr. Exp. Sta. Bull. 421.
1939. Bacterial wilt and ring rot disease of potatoes. In Proceedings of the Twenty-first Annual Conference Western Plant Quarantine Board. California Department of Agriculture. pp. 99-102.
1942. Further notes on the incubation period of the peach mosaic virus. Science 95: 256-257.

Examples of work done in collaboration with others:

Bodine, E. W. and L. W. Durrell. 1937. The Maynard plum, a carrier of the peach mosaic virus. Science 86: 81.
Kreutzer, W. A., E. W. Bodine, and L. W. Durrell. 1940. Cucurbit disease and rot of tomato fruit caused by Phytophthora capsici. Phytopathology 30: 972-976.
Bodine, E. W. and L. W. Durrell. 1941. Peach mosaic in Western Colorado (Abs.) Phytopathology 31: 4.
Bodine, E. W. and L. W. Durrell. 1941. Host range of peach mosaic virus in Western Colorado. Phytopathology 31: 322-333.
Bodine, E. W. and J. H. Newton. 1942. The rasp-leaf of cherry. Phytopathology 32: 333-335.
Bodine, E. W. and W. A. Kreutzer. 1942. Ring spot of apricot. Phytopathology 32: 179-181.
Simonds, A. O. and E. W. Bodine. 1943. A macrochemical reaction for the detection of peach mosaic. Science 97: 587-588.
Bodine, E. W. and E. L. Reeves. 1951. Ring pox of apricot. In virus diseases and other disorders with viruslike symptoms of stone fruits in North America. U.S. Dept. Agr. Handbook 10. pp. 187-188.

SUBCORTICAL MYCELIAL MATS AND PERITHECIA OF THE OAK WILT FUNGUS IN NATURE[1]

E. A. Curl, G. J. Stessel, and Bert M. Zuckerman

Visible growth[2] of the oak wilt fungus, *Chalara quercina* Henry, in nature outside of the wood of diseased trees, was first seen in Illinois[3] on September 1, 1951, in the form of a large subcortical mycelial mat.

During the summer and fall of 1950, at the beginning of a search for the way in which the oak wilt fungus becomes available for long distance spread, endoconidia were found in the frass in insect galleries under loose bark on wilt-killed trees in Illinois. Laboratory tests during the winter of 1950-1951 demonstrated that the fungus can grow to the surface of a diseased branch if the atmosphere is sufficiently humid. Sparse, but macroscopically visible, mycelium occasionally appeared on cut ends of branch pieces kept at 100 per cent humidity. Also, on several occasions endoconidia were found adjacent to knife wounds made through the bark, although no visible mycelium appeared.

METHODS OF FIELD STUDY.—Field studies were carried on from September, 1951, through May, 1952. These studies were made on oaks in 5 widely separated areas in Illinois: the Forest Preserve District of Cook County, Sinnissippi Forest in Ogle County, Detweiller Park in Peoria County, Payne Heath Woods in Piatt County, and Ross Woods in Vermilion County. No macroscopic growth of *C. quercina* was found on newly wilted and dying trees, as the bark remains tightly united with the wood for several weeks after the first symptoms appear. Therefore, most observations were made on trees on which the bark could be easily separated from the wood, and most attention was given to trees that had been dead from 2 months to 2 years.

Many standing trees were examined by removing bark from the lower portions of the trunks. Other trees were felled and examined by removing the bark from all portions of the trunks larger than 6 in. in diameter. Only red oaks (*Quercus borealis maxima* (Marsh.) Ashe) and black oaks (*Q. velutina* Lam.) were examined, as the bark of trees in the white oak group was either very tight or had reached the sloughing stage. The trees examined ranged in size from 10–24 in. in diameter at breast height.

SUBCORTICAL MATS OF CHALARA.—The first subcortical mycelial mats of *C. quercina* found in Illinois were on a red oak in the Forest Preserve District of Cook County. This tree showed the first symptoms of wilt in the early part of July, 1951, and was felled on August 2, 1951. Thirty days later, a mat of the fungus was found beneath the bark on the lower part of the trunk. Twelve more mats were found on this tree in late September when the bark was removed from the entire trunk.

A search for *Chalara* mats was then made in other oak wilt areas in Illinois. Fifteen of approximately 150 standing trees examined by removing the bark from the lower parts of the trunks showed visible mats of *C. quercina* (Fig. 1, A). Twelve other wilt-killed trees were felled and all bark was removed from the main trunks, 7 bore mats of the fungus in the subcortical region (Fig. 1, B). All trees that bore fresh subcortical mats had been dead for approximately 3 months to 1 year. The fungus was never found under the very tight bark of recently wilted trees, but black, dried mats partly overrun by saprophytic fungi were found on 2 trees estimated to have died 2 years previously.

Subcortical mats were present on the inner surface of the bark as well as on the surface of the wood. The number of separate mats found per tree varied from 1–13. These were located at distances of 2–20 ft. above ground.

The mats varied in shape and size from narrow individual areas measuring 2–15 cm. in length to areas irregular and broken or entire in outline measuring 2–20 cm. in width (Fig. 1, C). They consisted of a sparse to dense mycelial felt measuring an average of 1 mm. in depth. The color of the mat was light to dark grayish tan. New mycelial growth frequently appeared as irregular light gray areas over the surface or around borders of the mats. Water droplets usually dotted the surfaces of actively growing mats. A dark sclerotium-like pad was often present in the center of the mat.

The microscopic characteristics of young mycelial mats from nature were similar to those of the many cultures of *C. quercina* grown in our laboratory. Endoconidia were usually abundant and frequently occurred in chains. Pure cultures were obtained by direct transfers from mycelial mats on wood and bark of potato-dextrose agar. Conidiophores and endoconidia typical of *C. quercina* were produced abundantly in these cultures.

ASSOCIATED INSECTS.—The trunks of all trees on which mats were found had been extensively invaded by species of insects belonging to the families Scolytidae, Cerambycidae and Buprestidae.[4] Larvae of the

[1] Accepted for publication October 23, 1952.

This investigation was supported in part by grants from the Forest Preserve District of Cook County, Illinois, and the National Oak Wilt Research Committee.

[2] A photograph showing mycelial mats of *C. quercina* was displayed by the Department of Plant Pathology, University of Wisconsin, at the meeting of the North Central Division of the American Phytopathological Society at Madison, Wisconsin, in June, 1951.

[3] Curl, E. A., G. J. Stessel, and Bert M. Zuckerman. 1952. Macroscopic growth of the oak wilt fungus in nature. (Abs.) Phytopathology 42: 6.

[4] Insects were identified by Milton W. Sanderson, Associate Taxonomist, Illinois State Natural History Survey.

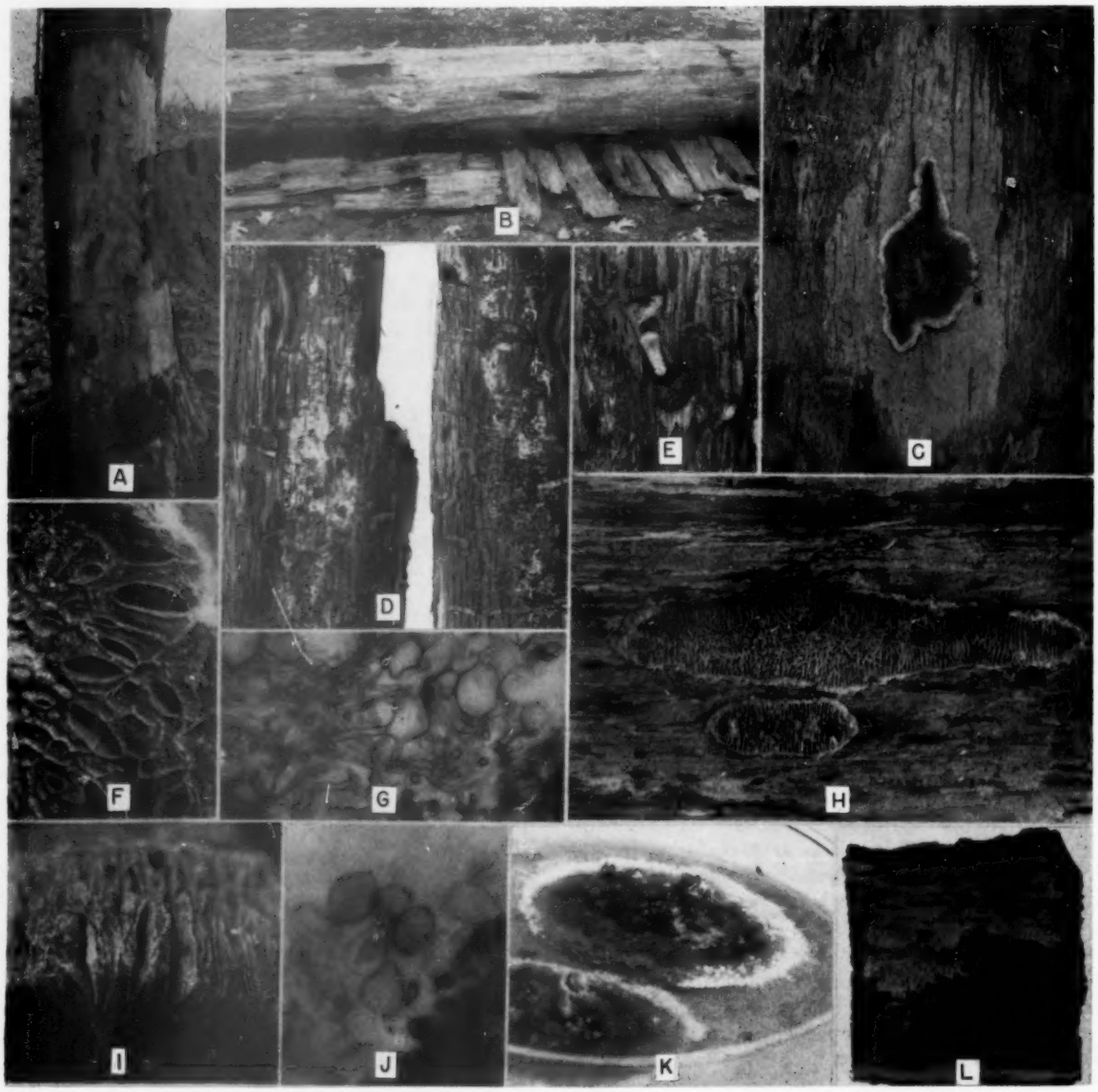


FIG. 1. Free-growing subcortical mycelium of *Chalara quercina*. A) Mycelial mats on the trunk of a red oak, with black pads marking their centers; B) mycelial mats (dark areas) on the surface of a peeled red oak log; C) mycelial mat (light gray area with a sclerotium-like pad in its center; D) mycelium growing over and among insect galleries; E) larva of the family Cerambycidae in its mycelium-filled inner bark chamber; F) surface of a partly dry pad, × 10, showing pits formed by the open rifts in the pad; G) interior of the pad, × 180, showing inflated cells, normal hyphae and, in the center, the connection between the two; H) sclerotium-like pads with daedaloid surface markings, × ⅔; I) edgewise view of a sliced pad, × 10, showing deep rifts below the daedaloid surface markings; J) spheroidal cells that compose the pad, × 180, showing loose cells at the tip of a rift; K) sclerotium-like structure produced by *C. quercina* on chestnut meal agar; L) mycelial growth formed on the inner surface of a piece of bark kept in high humidity.

2-lined chestnut borer, *Agrilus bilineatus* Weber, were especially numerous. In the early stages of wilt only occasional insect galleries and young larvae were seen under the still tight bark. Six weeks later, however, the number of galleries and larvae in the outer wood had increased greatly wherever the bark had become loose. In September, mycelium from mats of *C. quercina* was frequently seen growing in and across insect galleries (Fig. 1, D) and, in one instance, occupied the same chamber with an active larva of the family Cerambycidae (Fig. 1, E). Mycelium with abundant conidiophores and conidia was found in the outer portions of tunnels in which adults of the family Scolytidae were present. Four species of Buprestidae, 3 of Scolytidae, and 3 of Cerambycidae were frequently observed in association with mats of the oak wilt

fungus under the bark of wilt-killed trees.

THE SCLEROTIUM-LIKE STRUCTURE.—Dark pads centrally located in and much thicker than the mats were found on 20 of 22 trees (Fig. 1, C, H). Such pads were not present in the mats on the remaining 2 trees. The main body of the pad was usually almost free of visible loose mycelium, but hyphae frequently grew abundantly on the outer edges, forming a definite tan border around the pad.

These pads were irregular in outline, generally elongated oval to elliptical, 0.5–3 cm. wide, 2–10 cm. long, and 2–8 mm. thick. They were firm throughout but had a high water content. In color they ranged from dull green to black, according to their condition or age. Their surfaces were marked by vein-like networks of transverse striations that gave them an appearance rather aptly described by Leach *et al*[5] as daedaloid (Fig. 1, H). This network was composed not of ridges, but of loose cells, light in color, that marked the lips of deep, closed rifts in the structure (Fig. 1, J). The rifts, for the most part, extended completely through the structure (Fig. 1, I). When the pads dried out the sides of the rifts spread apart, making the surface of the structure rough and forming gaping holes (Fig. 1, F, I).

These pads were found at various times from October, 1951, to May, 1952, either under intact bark or directly beneath long narrow cracks in bulging bark. Pads located under cracks were sometimes soft and gave off an odor suggesting decayed organic matter. At this stage, various kinds of small flies and larvae were usually present. Upon exposure to air, fresh pads became dry and flat in 24 hour.

Unstained paraffin sections of the pad, cut at 13 and 15μ, showed that the pad is composed mainly of inflated, irregularly shaped but mostly ovoid to spheroidal brown-walled cells (Fig. 1, G, J). These cells are arranged in a pseudoparenchymatous manner and vary in size from 13 $\times$ 16μ–40 $\times$ 62μ. Numerous tan hyphae of normal diameter ramify among these cells, often swelling at their tips into larger and larger cells that merge among the cells that compose the pad (Fig. 1, G). Typical cultures of *C. quercina* developed on potato-dextrose agar from transfers made from either the surfaces or the interiors of young pads.

Somewhat similar sclerotium-like structures were induced *in vitro* in cultures of *C. quercina* (Fig. 1, K). The fungus was grown on chestnut meal agar (25 gm. ground Chinese chestnut meats and 20 gm. agar per liter) at 25°C. for 3 weeks and then placed at 8°C. The structures appeared in central portions and near the outer edges of cultures 6–8 weeks after seeding of the plates (3–5 weeks at 8°C.). They were irregular in shape, rather firm and dark or dull green. The largest one measured 0.5 $\times$ 2 cm. They consisted of cells corresponding closely in size, shape, and color to the cells composing the pads occurring in nature.

INDUCED MYCELIAL GROWTH.—Under certain environmental conditions, mats of *C. quercina* developed on pieces of bark and wood taken from wilt-killed trees. In September, 15 samples of bark and wood, showing no visible mycelium when taken from a diseased black oak, were kept moist in a closed metal box at field temperature. Dense, sporulating mycelial mats of the oak wilt fungus grew over the surfaces of the wood and inner bark in 36–48 hour (Fig. 1, L). Samples from healthy oaks and oaks showing very early symptoms produced no such growth when treated in the same way. Bark pieces that showed no visible mycelium were taken from the black oak mentioned above and placed on the forest floor, inner bark down. Thick sporulating mats of *C. quercina* developed on the inner faces of these pieces in 1 week. Similar results were obtained with bark and wood from a wilt-killed red oak.

PERITHECIA OF CHALARA QUERCINA IN NATURE.—Naturally produced perithecia of *C. quercina* were discovered on a wilt-killed red oak in Detweiller Park, Peoria, Illinois, on October 26, 1951. The tree measured 14 in. in diameter at the base and had been dead for approximately 3 months. The bark was bulged and cracked in 3 places on the lower part of the trunk. The cracks, all in vertical alignment on the trunk, were 2–10 in. in length and extended to the wood. Beneath each bulge was a space between wood and bark about 6 in. in width. A mycelial mat with the characteristic sclerotium-like pad in its center was found beneath each bulge. Numerous black perithecia were partly embedded in the mat within the lowest bulge. Later, perithecia were found beneath the other 2 cracks. Proof that these perithecia were the perfect stage of *C. quercina* will be given in another paper.

PATHOGENICITY TESTS.—Spore suspensions made from cultures started by transferring from naturally occurring mycelial mats to potato-dextrose agar proved pathogenic when injected into greenhouse-grown oak seedlings. Inoculated trees wilted in 3–6 weeks and the fungus was reisolated and identified. Cultures derived from the surfaces and interiors of sclerotium-like pads also were pathogenic to oak seedlings.

DISCUSSION.—The occurrence of numerous sporulating mats of *C. quercina* between the wood and bark of wilt-killed trees in widely separated counties in Illinois during the autumn and winter of 1951 and the spring of 1952 indicates that the fungus occurs frequently in this form in oak wilt areas. Endoconidia and ascospores produced in such mats could well serve as inoculum for spread of the pathogen to healthy oaks.

The almost constant presence of a sclerotium-like pad in the central portion of the mycelial mat suggests that it is a special structure produced by *C. quercina*. Its microscopic characteristics, the pathogenicity of cultures derived from it, and our production of a growth similar to it on artificial media support this view. Factors that stimulate development of this structure have not been determined fully, although low

[5] Leach, J. G., R. P. True and C. K. Dorsey. 1952. A mechanism for liberation of spores from beneath the bark and for diploidization in *Chalara quercina*. Phytopathology 42: 537-539.

temperatures may be important.

The watery nature of the pad, its central location in the mycelial mat, and its structure suggest 2 possible functions. One function may be to accumulate from the interior of the tree, store, and give out moisture, thus maintaining a humid atmosphere in which the mat can grow and produce spores. Another function may be to separate the bark from the wood mechanically, by the force it exerts in growing, thus creating a space or chamber in which the mat can develop. Also, by further increasing in thickness or extent, it may exert pressure enough to crack the bark.

It is imaginable, with the bark broken, that endoconidia and, if perithecia are present, ascospores can escape, that insects can enter to feed on the fungus or breed in it, and that endoconidia produced elsewhere can enter and fertilize the mat, causing the production of perithecia. It is imaginable, too, that separation of the bark from the wood and cracking of the bark begin a disintegration of the bark cover that eventually will expose the entire mat. Obvious though these possibilities are, there is no proof at the time of the writing of this paper that any of them actually takes place. Although pads and their surrounding mats appear more often than not beneath cracks, they frequently are found beneath unbroken bark. Moreover, disintegration of the dead cambium brings about the sloughing of bark from trees on which no pads or mats ever form.

The presence of fertile perithecia only in mats beneath cracks in the bark suggests that spermatization of the mats had taken place by air-borne or insect-borne spores from other mats of compatible lines.

The insects that were observed in the subcortical regions of trees bearing the mycelial mats were by no means all of the species of insects known to infest dying and dead oak trees. However, the abundance under the bark of larvae of Cerambycidae and Buprestidae and adults of Scolytidae seems worth consideration in searching for possible vectors. As these bark beetles have the opportunity to be intimately associated with the fungus, transportation of spores by emerging insects of these families appears to be possible. *Agrilus bilineatus* Weber, for example, emerges from dead trees in the spring and feeds on leaves of healthy oak trees[6].

[6] Chapman, Royal N. 1915. Observations on the life history of *Agrilus bilineatus*. Jour. Agr. Res. [U. S.] 3: 283-294.

The rapid development of sporulating mats of *C. quercina* on wood and bark pieces from wilted trees when these pieces lie on the forest floor should be considered in the planning of control measures based on sanitation, as mats developing in this manner might furnish additional inoculum in oak wilt areas. Also, this phenomenon raises the question as to whether the oak wilt fungus can be transferred in nature to trees that have been killed or weakened by various causes and there develop and persist saprophytically.

SUMMARY

Visible growth of the oak wilt fungus, *Chalara quercina* Henry, was found in Illinois as large subcortical mycelial mats on 22 wilt-killed oak trees during the autumn and winter of 1951 and the spring of 1952. The mats were found only under loose bark of wilt-killed trees and present on the inner surface of the bark as well as on the surface of the wood.

Species of insects belonging to the families Scolytidae, Cerambycidae and Buprestidae were found beneath the bark of all trees having mycelial mats. Larvae and adults of bark beetles were observed in direct contact with the fungus.

Sclerotium-like pads were present in the centers of mycelial mats on 20 of the 22 trees bearing mats. The pads usually were located directly beneath cracks in the bark. A structure similar to the pad found in nature was produced *in vitro* by cultures of *C. quercina* held at a low temperature.

Pure cultures, in all respects typical of *C. quercina*, were obtained by transferring directly from mycelial mats and from sclerotium-like pads and proved pathogenic when inoculated into seedling oaks.

Pieces of bark and wood that showed no visible mycelium when taken from wilted red and black oaks quickly developed large sporulating mats of the oak wilt fungus when kept in a moist box or allowed to lie on the forest floor.

Perithecia of *C. quercina* were discovered on a wilt-killed red oak in Illinois on October 26, 1951. They were present in large numbers on mycelial mats located beneath cracks in the bark.

Section of Applied Botany and Plant Pathology
Illinois State Natural History Survey
Urbana, Illinois

THE PERITHECIAL STAGE OF CHALARA QUERCINA IN NATURE [1]

G. J. Stessel and Bert M. Zuckerman

Prior to 1951, knowledge of the life history of the oak wilt fungus consisted primarily of a description of the imperfect stage of the causal organism, *Chalara quercina* Henry, and proof of pathogenicity of the fungus, reported in 1944 (4). Bretz (1) reported production of perithecia by paired cultures of this fungus in 1951. Recently, Curl, Stessel, and Zuckerman (2,3) reported that the oak wilt fungus develops in the form of macroscopic, sporulating mats of mycelium between the wood and loosened bark of wilt-killed trees. Later, Hepting, Toole, and Boyce (5) reported the production of perithecia of *C. quercina* in cultures isolated from a single wilted tree.

On October 26, 1951, perithecia[2] of *C. quercina* naturally produced on a wilted oak were found in Detweiller Park, Peoria, Illinois. The tree on which the perithecia developed was a red oak (*Quercus borealis maxima* (Marsh.) Ashe) that had died from wilt 2 or 3 months earlier. The tree had 3 cracks in the bark of the lower trunk. These cracks were several inches long, extended inward to the wood, and occurred at distances of 2, 6, and 10 ft. above the ground. The bark surrounding each crack had loosened and formed an oval-shaped bulge (Fig. 1, A). Removal of the bulging bark surrounding the lowermost crack revealed 2 large, greenish, gelatinous, sclerotium-like pads each surrounded by a wide mycelial mat of *C. quercina*[3]. One mat was on the outer surface of the wood, the other on the inner surface of the bark, and the two grew as mirror images of each other. The mats measured 10 × 20 cm. and the pads 2 × 6 cm. (Fig. 1, B). A smaller pad was located several cm. to one side of the larger pad on the wood and inner bark surfaces. The mats and surrounding wood and bark were removed and stored at 8°C. for subsequent laboratory studies. The dark tan, velvety mats contained numerous conidiophores and endoconidia characteristic of *C. quercina*. Many perithecia were partly embedded in the mats and along the borders of the pads. Transfers of spores and mycelium from these mats to potato-dextrose agar produced cultures that were identical with *C. quercina* in color and texture. These cultures produced endoconidia within 9 days at 25°C.

THE PERITHECIAL STAGE.—*Materials and methods.*—Perithecia found in nature and their contents were studied under low magnification, then in unstained water mounts, in lacto-phenol mounts stained with cotton blue as prepared by Riddell (8), and in stained sections. In preparing sections, small portions of fungus mat containing perithecia were fixed in formalin-aceto-alcohol, aspirated, dehydrated with ethyl alcohol, and cleared with xylol (9). In later work clearing was done with cedar oil in place of xylol (7). The material was embedded in paraffin and sections were cut at 13–15μ. Sections were stained by several methods. Best differentiation of perithecial contents was obtained with Pianeze III B (10).

Germination studies were made with ascospores taken directly from perithecia 1–2 weeks after the perithecia had been removed from the tree. Spores were placed in drops of nutrient solutions on flat and in deep-well slides and incubated at 25° in a saturated atmosphere. Nutrient solutions used were 1) dextrose-mineral salt solution, 2) 1 per cent dextrose solution, 3) 2 per cent dextrose solution, and 4) 25 per cent orange juice solution. The dextrose-mineral salt solution contained (per liter) dextrose 1 gm., KNO_3 0.1 gm., KH_2PO_4 0.14 gm., and $MgSO_4.7H_2O$ 0.5 gm.; the pH was 5.5.

Two types of chestnut agar, chestnut meal-extract agar and chestnut meal agar, containing (per liter) Chinese chestnut meats 85 gm., and agar 17 gm., pH 5.5–5.6, were used in the study of heterothallism of single ascospore isolates. In the former type of medium a boiling water extract of the chestnut meats was used to make the medium, in the latter type boiled, ground chestnut meats were used.

Description of the perithecial stage.—Perithecia on the tree were barely discernible to the unaided eye. On magnification they appeared as dark brown to black, flask-shaped, solitary bodies. They were abundant, with as many as 5 per sq. mm. (Fig. 1, C). Their spherical to slightly flattened bodies were almost entirely embedded in the fungus mat. The beaks were as long as or slightly longer than the diameter of the perithecial bodies. When collected, mats were moist and the perithecial beaks were flared in a funnel-shaped manner, narrow at the base and progressively wider toward the top. From 1–2 weeks after collection, the hyphae composing each beak had gathered together to form a compact, tapering beak with a well defined ostiole (Fig. 1, C). Observation of these perithecia, and others found later, showed that the beaks were composed of erect, parallel hyphae tightly compressed at the base, loose at the top, and hygroscopic, the beaks flaring under moist conditions and contracting under drier conditions. When collected many perithecia showed sticky, creamy exudates at the ostioles (Fig. 1, E). Occasionally the exudates extended part way down the outside of beaks. These exudates were com-

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This investigation was supported in part by grants from the Forest Preserve District of Cook County, Illinois, and the National Oak Wilt Research Committee.

[2] Photographs of these perithecia were exhibited publicly at the annual meeting of the American Phytopathological Society at Cincinnati, Ohio, during the session of Section A, Oak Wilt, on December 11, 1951.

[3] In this paper the term pad refers to the thick, gelatinous growth found in central portions of mycelial mats, and the term mat refers to the sporulating, felt-like mat of mycelium found between the wood and bark of wilted trees.

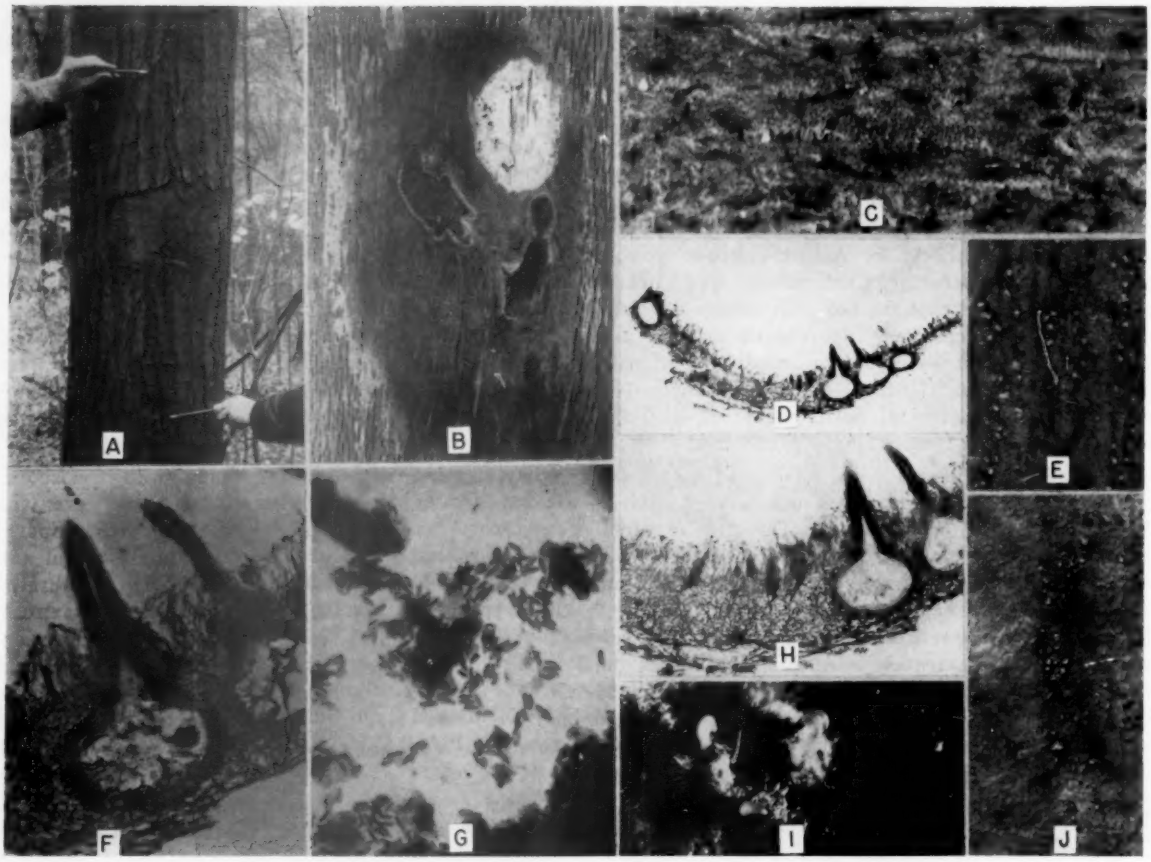

FIG. 1. A) Bulging, cracked bark of red oak, beneath which perithecia of *C. quercina* were found. B) Subcortical mat containing numerous perithecia and centrally located gelatinous pads found at lower location pointed out in A. C) Short, black beaks of perithecia protrude above the mycelial mat. D) Cross section of mat showing thickness of stromatic portion containing perithecia. E) Naturally occurring perithecia of *C. quercina*, showing ascospore discharge at time of collection. F) Cross section of perithecia embedded in fungus mat obtained in nature, ×90. G) Ascospores lying free in perithecial cavity, ×600. H) Detail of thickened portion of mat wherein perithecia are produced. I) Typical extrusion of ascospores in a sticky matrix from perithecia produced in culture. J) Perithecia produced in a pairing of compatible single ascospore isolates on chestnut meal agar.

posed of ascospores in a sticky matrix that was not readily soluble in water. When the necks and upper portions of perithecia were removed the contents of the perithecia were usually wet, sticky masses filling the interior. In some perithecia the contents were dry, wax-like masses that partly filled the interior. A few perithecia were empty. Wet mounts of crushed perithecia showed an abundance of ascospores that occurred singly, in pairs, in groups of 6 or 8 or in large aggregates. Ascospores were hyaline, 1-celled, and elliptical in shape with 1 face flattened or curved slightly inward. Portions of the mycelial mat and enclosed perithecia have been preserved.[4]

The morphology of the perfect stage structures observed in stained sections was in agreement with that described above, and agreed, within limits, with the description given by Bretz (1). Measurements of perithecia, asci, and ascospores are in Table 1. These are measurements of untreated and of stained, sectioned material from the tree and of perithecia produced in culture by pairing compatible isolates of the fungus. Measurements of the perfect stage structures reported by Bretz (1) are included.

Measurements were made on longitudinal sections of 9 perithecia. These median sections were cut through the ostioles (Fig. 1, F). Mature perithecia in the sectioned material varied considerably in size. Perithecia were in 3 different stages of development: those that did not contain ascospores, those that contained ascospores and asci, and those that contained masses of ascospores only. Perithecia devoid of ascospores contained sac-like structures attached to the inner faces of perithecial walls by long strands. These strands appeared to have originated at the inner wall of the perithecium and grown toward the center of the perithecial cavity. The sac-like structures contained 1 or more nuclei and were interpreted as early stages of ascus

[4] This material has been deposited as Accession No. 31357 in the Mycological Collection of the Illinois State Natural History Survey, Urbana, Illinois.

TABLE 1.—*Measurements in microns of perithecial structures of C. quercina found in nature and developed in culture*

Structure	Reported by Bretz (1) *in vitro*	Developed *in vitro*[a]	Perfect stage found in nature[b]	
			Fresh material[c]	Stained sections
Perithecia, width	240–380	.....	.....	266–380
Beaks, length	250–450	240–420	.....	213–400
Asci, maximum diameter	7–10	.....	.....	8.5–9.5
Ascospores				
width	2–5	1.0–4.8	0.9–3.4	1.0–4.0
length	3–10	2.4–6.5	2.8–6.9	2.3–7.8

[a] Perfect stage structures developed in this laboratory by pairing stock cultures of *C. quercina.*
[b] Perithecia stored 1–2 weeks at 8° after collection from a wilted red oak.
[c] Observed in wet mounts.

development. Portions of the perithecial cavity not occupied by these structures were filled with a deep-staining matrix. Perithecia in a more advanced stage of growth contained globose to subglobose asci that lay free in the perithecial cavity. The limited number of asci found in these sections indicates that they are ephemeral and, as Bretz (1) suggested, evanescent. These asci contained clearly defined spores arranged in a circular pattern within the asci. The number of spores in an ascus could not be determined with certainty. As many as 6 spores were seen in some asci, but the number is probably 8. Spores were frequently arranged in pairs in the masses of ascospores lying free in the deep-staining matrix (Fig. 1, G).

The structure of the mycelial mat, perithecial bodies, and beaks of perithecia was studied from stained sections. Portions of the mat that bore perithecia were much thicker and more compact than contiguous portions in which no perithecia developed (Fig. 1, D). Perithecia were embedded in thick, stromatic portions of the mat, the hyphae of which were very irregular in width and closely interwoven. These stromatic portions definitely are not identical in structure with the pads; they are a plectenchyma consisting of narrow interwoven hyphal strands that form a moderately dense prosenchyma (Fig. 1, F, H). Pseudoparenchymatous tissue was not present in these stromatic portions. A thinner layer of loose, felt-like erect hyphae and conidiophores surmounted all portions of the mat (Fig. 1, D, F, H). The beaks consisted of compacted, parallel hyphae continuous with the hyphae composing the upper portion of the perithecial bodies (Fig. 1, F). The walls of the perithecia were clearly distinct from the surrounding prosenchyma because the interweaving of the hyphae composing them was more dense. However, these walls were intimately connected with the prosenchyma, the hyphae of which actually formed the outer perithecial wall (Fig. 1, F, H).

Ascospores obtained from perithecia produced in nature germinated in 8–12 hours in the dextrose-mineral salt solution and in 12 to 18 hours in the other 3 solutions. Germ tubes grew to about 4 times the length of the spores in 8–12 hours in the dextrose-mineral salt solution and usually doubled this length at the end of 18 hours. Germ tubes grew rapidly in all solutions during the interval 24–36 hours. The germinated ascospores swelled to a fully elliptical shape during this interval and were usually 1½–2 times the size of the ungerminated spores. Approximately 30 per cent of the spores germinated in 2 per cent dextrose solution. Germination in the other solutions varied from 20–30 per cent. Spores produced single germ tubes, nearly always polar but sometimes subpolar in position. The germ tubes were non-septate, except as endoconidia formed. Endoconidium production was abundant in germ tubes and in hyphal branches during the interval 24–36 hr. in all solutions. The endoconidia measured 1.9–3.5 × 6.3–10.6μ.

Heterothallism.—Single ascospore cultures were used to study the sexual characteristics of the fungus. Masses of ascospores obtained from the perithecia produced on the tree were distributed in drops of 2 per cent dextrose solution. Ascospores from individual perithecia were placed in separate drops. Separation of ascospores was difficult because the spores tended to remain in groups in the sticky matrix. The drops were then streaked on clear agar (6), incubated at 25°, and observed for germination. Isolated groups of 3 or more germinating ascospores obtained from a single perithecium were transferred to potato-dextrose agar. In each culture that developed, perithecia appeared in 10 days to 2 weeks. The beaks of these perithecia were flared and sticky masses of ascospores oozed from them. Ascospores germinated in 12–18 hours in dextrose solution and produced endoconidia abundantly in 18–24 hours.

From a perithecium, chosen at random, that had developed in a culture originating from 3 ascospore sporelings as described above, ascospores were obtained and plated on clear agar (6) at 25°. After germination, but before endoconidium production, single sporelings were transferred to plates of chestnut meal-extract agar. Nine of these sporelings developed into cultures. Each isolate was characteristic of *C. quercina* in appearance and sporulation. Eight isolates were paired in all possible combinations and each combination was made in triplicate on both types of chestnut agars. On chestnut meal-extract agar, mycelial growth on small agar blocks was placed about 2 cm. apart. On chestnut meal agar, spores in suspension were streaked about 2 cm. apart. Results of the pairings of the eight isolates are in Table 2.

The 8 isolates fell into 2 groups for sexual compatibility, 5 isolates in one group and 3 in the other. All intergroup pairings produced fertile perithecia in 2–3 weeks, in the area where 2 cultures met (Fig. 1, I, J). No intra-group pairings produced perithecia in 4 weeks. These results indicate that the fungus is

TABLE 2.—*Production of perithecia in pairings of 8 single ascospore isolates of C. quercina in culture.*[a] *Isolates were derived from a culture that developed when 3 ascospores from a perithecium found in nature were cultured*

Isolate number	Isolate number							
	M1	M2	M7	M12	M13	M3	M6	M9
M1	O	O	O	O	O	+	+	+
M2		O	O	O	O	+	+	+
M7			O	O	O	+	+	+
M12				O	O	+	+	+
M13					O	+	+	+
M3						O	O	O
M6							O	O
M9								O

[a] + = Perithecia. O = No perithecia.

heterothallic in sexual characteristics. Results of pairings were the same on both media. However, perithecia were produced more abundantly on chestnut meal agar than on chestnut meal-extract agar. It was also found that when compatible single ascospore cultures were paired on potato-dextrose agar in place of chestnut meal agar, fertile perithecia were produced.

Two of the compatible single ascospore isolates were paired with each of 2 compatible conidium isolates. The single conidium isolates, I and II, originated from cultures previously isolated from wilting oaks and were pathogenic. Results of these pairings on chestnut meal-extract agar are in Table 3.

To test whether the fungus mat that developed perithecia in nature was composed of plus and minus thalli, 4 cultures made from the outer border of the mat and 1 culture made from the pad were paired in all possible combinations with 5 isolates of known plus and minus relationships that had been obtained from other wilted trees. The 5 cultures obtained directly from the mat and pad fell into 1 group for sexual compatibility; all were self-incompatible. In this limited test only 1 line of the fungus was isolated as a result of the 5 mass transfers from the mat and pad.

Observations and studies during winter and spring.—The occurrence of open cracks in bark bulges directly over subcortical mats and perithecia of the oak wilt fungus suggested that conidia and ascospores might escape from the trunk and be distributed by wind. Attempts were made at irregular intervals during December, 1951, and January and February, 1952, to trap endoconidia and ascospores that might be liberated through the cracks. Slight suction was applied to the cracks in some tests. Spores that had the appearance of endoconidia of *C. quercina* were obtained but failed to grow.

TABLE 3.—*Production of perithecia in pairings of 2 single ascospore isolates with single conidium isolates I and II in vitro*[a]

Single Conidium isolate	Single Ascospore isolate	
	M3	M7
I	+	O
II	O	+

[a] + = Perithecia. O = No perithecia.

The trunk of the wilted red oak in Detweiller Park was examined further during the winter and spring of 1952. Bulging bark at the second crack above the ground and the underlying wood were examined during a warm period of high relative humidity in late January and early February, 1952. The large mats found between the bark and wood had large, black sclerotium-like pads in the centers. These mats, similar to those collected in October, 1951, bore numerous perithecia, most of which were extruding sticky masses of ascospores. Some perithecia, however, contained dry, wax-like masses in which ascospores were embedded, while others were empty. Ascospores from the extruding masses and from the wax-like masses germinated readily in 2 per cent dextrose solution, and germ tubes produced endoconidia abundantly in 24–36 hours.

The mats collected on October 26, 1951, were reexamined after storage at 8° for 4 months. The mycelium was partly dehydrated and beaks of the perithecia were hard and contracted. Portions of these mats were incubated in a moist chamber at 22°. Within 9 hours, the mycelium became moist and new conidiophores and conidia developed. Beaks of the perithecia had also become moist and the hyphae at their tips had expanded outwardly. Beaks became flared at the end of 48 hours. In 48 to 60 hours they became surmounted by small, circular, convex, dense tufts of pale tan, velvety mycelium. The tufts contained numerous conidiophores that produced endoconidia in long and short chains. During this interval, similar tufts of mycelium appeared at random on the previously dry mat and on the central pad.

The bulging bark at the third crack above the ground and the wood beneath it were examined during the last week in April, 1952. A tan mat, 6 × 10 cm., with a centrally placed pad was found under the bark. The mat was damp and partly decomposed. Numerous adult insects (springtails) were working actively in the mat and a large number of small roundworms (nematodes) were present. Leathery, moist, and partly collapsed perithecia were abundant. All perithecia examined were devoid of ascospores. The beaks were erect and contracted; ostioles were clearly defined. Wet mounts of material from this sample contained neither conidia nor ascopores. A number of long-beaked perithecia, different from any previously found, and several types of coremia were present in various portions of this particular mat.

PATHOGENICITY TESTS.—Young oak trees in the greenhouse were used in pathogenicity studies. Inocula were ascospores in perithecial exudates obtained directly from the wilted red oak in Peoria in February, 1952, dry wax-like masses of ascospores taken from the interiors of perithecia that had been stored for 4 months at 8°, single ascospore isolates M3 and M7, and both single conidium and mycelial isolates derived

from the mat and pad collected on October 26, 1951. The inoculated trees showed typical wilt symptoms in 4–6 weeks. The fungus reisolated from each of the wilted trees was identified as *C. quercina*.

ADDITIONAL TREES ON WHICH PERITHECIA WERE FOUND.—In the latter part of March, 1952, large mats were found on a red oak near White Heath, Piatt County, Illinois. Although the overlying bark was not cracked, these mats had the usual pads in their centers. When this material was collected it bore neither perithecia nor endoconidia. Bark and wood pieces enclosing mats were placed in a moist chamber at 21°. Perithecia exuding ascospores, similar to those found in Peoria in October, 1951, were present 2 days later. The ascospores germinated in dextrose solution and germ tubes produced endoconidia.

Perithecia were found in typical mats on 7 red oaks in Detweiller Park during late April and early May, 1952. Bark cracks occurred over each of the subcortical growths on these trees. Ascopore masses visible to the unaided eye crowned the perithecial beaks on 6 trees. On the other tree the perithecial beaks had been partially eaten away by scavenger insects. Ascospores from the 6 trees germinated in dextrose solution and the germ tubes produced endoconidia. Also, ascospores from each of the 6 trees developed into typical *C. quercina* cultures.

INSECT OBSERVATIONS.—Larvae of the families Buprestidae and Cerambycidae and adults of the family Scolytidae have been found in galleries underlying or adjacent to sporulating mats (2, 3). Mats containing perithecia were examined for insects. Several adult beetles, genus *Rhizophagus*, and numerous adult springtails, *Isotoma cinerea* Nicolet, were present in and on the mat found on the red oak in Peoria, in February, 1952.[5] The springtails were working actively among the perithecia and a number had their mouth parts embedded in the perithecial exudates. Cages attached adjacent to, but not covering, bark cracks within which were perithecia, captured a number of adults of the family Scolytidae in April and May, 1952.

During late March, 1952, several beetles, genus *Rhizophagus*, were collected on the mat found under unbroken bark at White Heath. A small, circular hole, clearly visible in the mat, opened into a gallery in the underlying wood, similar to galleries made by Scolytidae. The mats with fertile perithecia found in fresh condition under bark cracks in Peoria in early May, 1952, harbored a number of insects of scavenger types. Larvae, genus *Agrilus* (Buprestidae), were found in subcortical galleries near these mats.

DISCUSSION.—The discovery of fertile perithecia, since proved to be the perfect stage of *C. quercina*, in subcortical mats of the oak wilt fungus on an oak tree on October 26, 1951, is the first finding known to the writers of the perithecial stage of *C. quercina* as it develops in nature. Furthermore, the presence of open cracks in the bark of this wilt-killed tree directly over fertile mats and perithecia is the first observation known to the writers of direct exposure of viable ascospores and endoconidia of the oak wilt fungus to the air. Similar mats and perithecia were present under the cracks in bulging bark of wilted trees found in May, 1952. The association of certain bark beetles and scavenger type insects with these fertile mats of *C. quercina* suggests that some of these insects may be agents of dissemination of spores. As the beetles have been found in close association with both spore forms of the fungus in nature and oak wilt results after each of these spore forms is placed in wounds of healthy oaks, the role of certain bark beetles as possible vectors of the pathogen and as disseminators of the spores needs intensive investigation.

The condition of mycelial mats and fertile perithecia found on wilted trees in Illinois during 1951 and 1952 indicates that viable ascospores in perithecial exudates are continuously available for distribution from October to May. Whether or not ascospores are discharged during the period from May to October has not yet been determined. The occurrence of new mycelial growth, endoconidium production on mycelial mats, and discharge of ascospores from perithecia after refrigeration of samples for 4 months suggest that mats and perithecia of *C. quercina* on wilted trees may remain viable over winter.

[5] Insects were identified by Milton W. Sanderson, Associate Taxonomist, and W. R. Richards, Laboratory Assistant, of the Illinois State Natural History Survey.

SUMMARY

Fertile perithecia of *Chalara quercina* were found on October 26, 1951, on a wilted red oak in Detweiller Park, Peoria, Illinois. These perithecia were embedded in subcortical mats of the fungus that had developed on the surface of the sapwood and inner surface of the bark. The central portion of each mat was occupied by thick, gelatinous pads. The mats and pads on wood and bark surfaces formed mirror images with each other. The bark overlying these mats was bulged and cracked through to the cambial region, exposing mats, endoconidia, and ascospores to the air. Ascospores taken from perithecia in these mats germinated readily and the germ tubes produced endoconidia. When groups of 3 or more ascospore sporelings were transferred to potato-dextrose agar fertile perithecia were produced in 10 days–2 weeks.

Of 8 single ascospore isolates tested, all were self-sterile. They comprised 2 groups for sexual compatibility, and were intra-group incompatible and inter-group compatible. Compatibility was determined by the production of fertile perithecia in the area where 2 cultures met. Single ascospore isolates paired with single conidium isolates of known pathogenicity produced perithecia in all pairings of plus with minus isolates.

Trees inoculated with single conidium isolates from the mycelial mat, masses of ascospores from perithecia found in nature, and single ascospore cultures derived from a perithecium obtained in nature, showed typical

oak wilt symptoms in 3–6 weeks. The fungus was reisolated from the wilted trees and identified.

On the basis of morphological measurements, ascospore germination studies, heterothallism tests, and pathogenicity trials, it was concluded that the perithecia as reported here are the perfect stage of *C. quercina* in nature.

Similar endoconidium-producing mats containing many fertile perithecia were found under bulging and cracked bark of 6 additional wilt-killed red oaks in Peoria, Illinois, in late April and early May, 1952. When found, the perithecia in these mats were exuding sticky masses of ascospores.

SECTION OF APPLIED BOTANY AND PLANT PATHOLOGY
ILLINOIS STATE NATURAL HISTORY SURVEY
URBANA, ILLINOIS

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PRESSURE EFFECTS IN FUNGUS AND VIRUS INFECTIONS[1]

C. E. Yarwood

In routine inoculations with bean rust, 2 pots accidentally tipped over while being removed from the incubation chamber, and a portion of an inoculated leaf was crushed between the pots. Rust development on this crushed leaf was inhibited for about a cm. beyond the region of visible injury. This led to a brief investigation of the effect of leaf pressure on infection with some plant pathogens, chiefly bean rust and tobacco mosaic virus.

METHODS.—Bean rust (*Uromyces phaseoli* (Pers.) Wint.), snapdragon rust (*Puccinia antirrhini* D. and H.), sunflower rust (*Puccinia helianthi* Schw.), spinach downy mildew (*Peronospora effusa* (Grev.) Rabh.), bean powdery mildew (*Erysiphe polygoni* DC), bean anthracnose (*Colletotrichum lindemutheanum* (Sacc. and Magn.) Briosi and Cav.), tobacco mosaic virus (TMV), and tobacco ringspot virus were maintained in the greenhouse by conventional methods. Inoculations with bean rust or anthracnose were made by applying a water suspension of uredospores or conidia to the lower surface of primary leaves with a pressure atomizer; the plants were then incubated overnight in a moist chamber before they were returned to the greenhouse bench. Inoculation with powdery mildew was made by dusting dry conidia on dry leaves; the plants were then left continuously on the greenhouse bench. With bean powdery mildew and bean rust the inoculations were made on Pinto bean. With bean anthracnose, trials of pressure effects were made on Pinto but the fungus was maintained on the more susceptible Bountiful bean.

Inoculations with TMV, tobacco ring spot virus, and tobacco necrosis virus were made by applying a water suspension of ground infected leaf tissue to the upper surface of primary Pinto bean leaves by means of a stiff brush. In most virus trials the leaves were dusted with carborundum before inoculation and the inoculum was suspended in 0.5 or 1 per cent K_2HPO_4. Inoculum of TMV was taken from systemically infected tobacco, and inocula of tobacco ring spot virus and tobacco necrosis virus were taken from bean.

In initial trials of pressure, relatively unknown and uncontrolled pressures were produced by pressing the leaf between thumb and forefinger. This produced effects similar to those to be described. Controlled pressure was produced with a Model PB3 Spring tester made by the Link Engineering Co., Detroit, Michigan. The compression scale bar was removed and 2 rubber stoppers with an exposed face of 1 sq. in. were cemented to the upper and lower steel jaws of the tester so that the faces of the stoppers coincided when compressed. When applied in this way pressures of over 50 lb. per sq. in. tore the leaves at the periphery of the treated area. To avoid this, paper caps slightly larger than the surface of the stoppers were cemented over the faces of the rubber stoppers, or the leaf was held between 2 pieces of paper during treatment. In test treatments the leaf was held flat on the lower stopper and the tester compressed slowly till the desired pressure was indicated on the scale, held for about 1 second, and released. Pressures over approximately 40 lb. produced a water-soaked appearance of the treated area, but the water soaking disappeared in a few minutes in the greenhouse.

Infection with rust, anthraconose and viruses was

[1] Accepted for publication October 24, 1952.

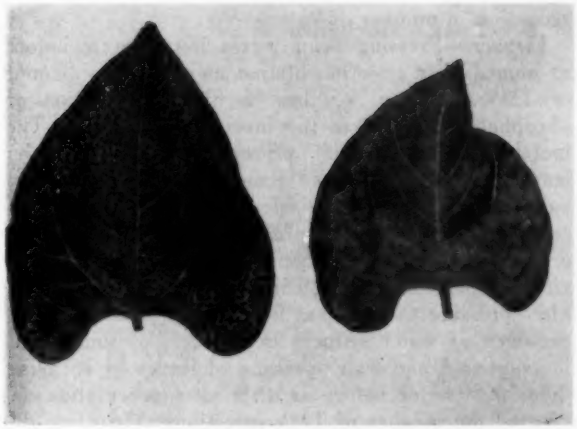

FIG. 1. Pressure therapy for bean rust and bean powdery mildew. Left. The bean leaf was pressed at 80 lb. per sq. in. in the 2 circles at 13 hours after inoculation with rust; these circles are practically free of rust pustules. Right. The bean leaf was pressed at 80 lb. per sq. in. in the center circle at 24 hours after inoculation with powdery mildew. The pressure treatment has greatly reduced mildew development. Photographed by L. V. Messier at 10 days after inoculation.

measured by counting the number of lesions. Infection with powdery mildew was estimated on a decimal scale in which 0 indicated no infection and 10 the heaviest infection. Leaf injury from pressure treatments was estimated on a decimal scale in which 0 indicated no injury and 10 indicated that the treated portion was killed.

RESULTS.—*Rusts.*—Pressure applied to bean leaves at 12–48 hours after inoculation with rust greatly reduced the number of pustules which subsequently developed (Figures 1, 2). Inhibition of lesion formation (pressure therapy) increased with increased pressure, and decreased with increased age of leaf and of infection. Leaf injury increased with increased pressure and decreased with increased age of the leaves. Pressure after inoculation was also effective in killing

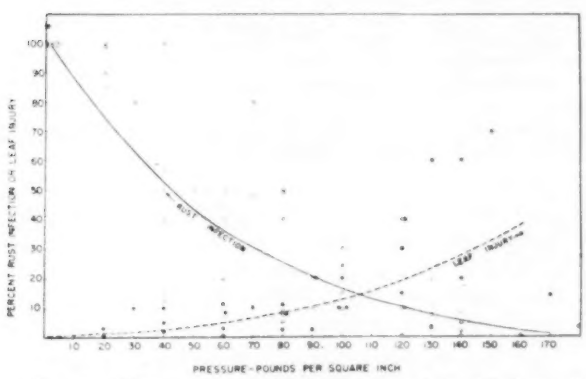

FIG. 2. Effect on rust development and leaf injury of pressure treatments at 16 hours after inoculation with rust. As the pressure was increased rust infection decreased and leaf injury increased. Rust infection or leaf injury was calculated as a percentage of the maximum observed in these tests.

young infections of sunflower rust and snapdragon rust. Areas where rust infections had been killed by pressing were completely susceptible to re-inoculation with the same rust. When bean leaves were pressed immediately before inoculation with rust, the treatment reduced infection, but if inoculation followed pressing after more than about an hour, there was usually little apparent effect of the treatment. This short-time protection from rust infection by pressing the leaves was probably due to the water soaking, from which the leaves soon recovered, since Cohen[2] has reported that

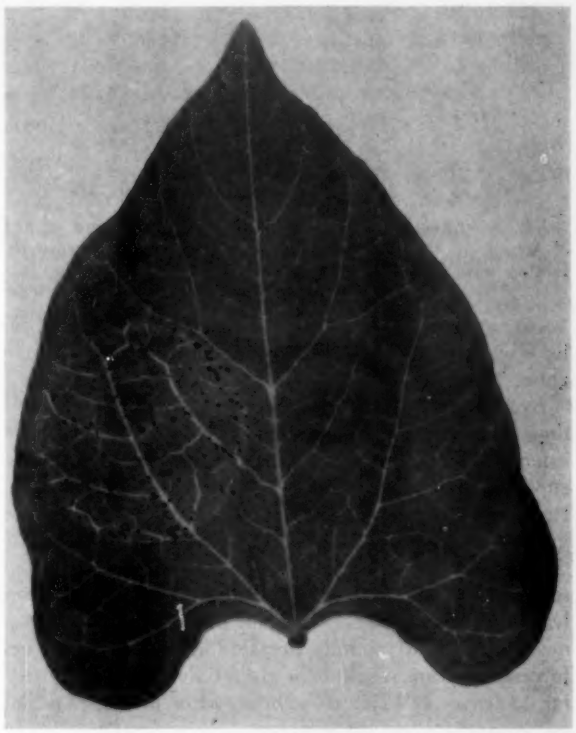

FIG. 3. Effect of pressure treatment on the susceptibility of a Pinto bean leaf to tobacco mosaic virus. The circular area (showing necrotic local lesions) on the left half of the leaf was treated with 70 lb. pressure, and the entire leaf inoculated with tobacco mosaic virus. This leaf is from a plant inoculated 16 days after seeding, and in the absence of pressure treatment, was highly resistant to tobacco mosaic virus. Inoculated Jan. 31, photographed Feb. 5, 1951. Photograph by L. V. Messier.

water-soaked bean leaves were resistant to rust infection. In the present study water soaking of leaves was readily induced by spraying water on the lower leaf surfaces. This treatment applied to young infections prevented many pustules from appearing, but was not so effective as mechanical pressure.

Bean powdery mildew.—Pressing of healthy bean leaves before inoculation had no clear effect on their susceptibility to powdery mildew. Pressing of leaves 1–6 days after inoculation reduced mildew development in all cases (Figure 1). In 4 trials, pressure at

[2] Cohen, Morris. 1951. Increased resistance to bean rust associated with water infiltration. Phytopathology 41: 937.

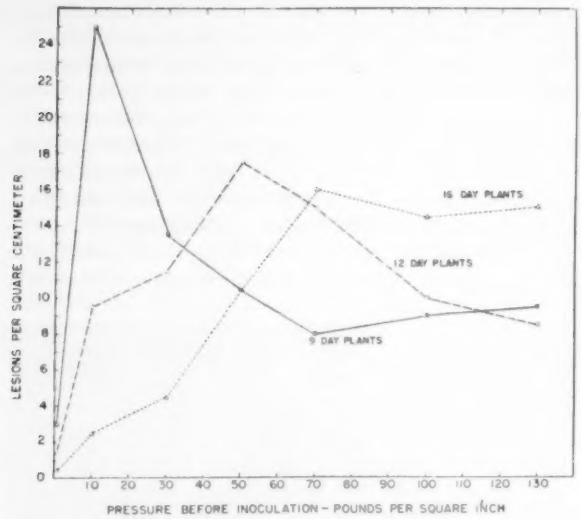
LESIONS PER SQUARE CENTIMETER
PRESSURE BEFORE INOCULATION – POUNDS PER SQUARE INCH
15 DAY PLANTS
12 DAY PLANTS
9 DAY PLANTS

FIG. 4. Effect of pressure before inoculation and of age of leaves on the susceptibility of Pinto bean leaves to infection with TMV.

10 lb. gave about 7 per cent reduction in mildew, 30 lb. gave about 25 per cent reduction, and 50 lb. gave about 65 per cent reduction.

Bean anthracnose.—Primary leaves of plants less than 8 days old were highly susceptible to anthracnose infection, and no specific effect of pressure on the susceptibility of these young leaves was demonstrated. Pressing of leaves of plants 10 days or more of age greatly increased their susceptibility. On 12-day plants the greatest increase in susceptibility resulted from treatment at about 20 lb. pressure. Untreated primary leaves of 15-day plants were not infected with anthracnose but 20–110 pounds pressure on these leaves before inoculation resulted in anthracnose lesions. Pressing of leaves at 12 or more hours after inoculation had no apparent effect on infection.

Spinach downy mildew.—In 1 trial with spinach downy mildew, plants were inoculated at 5 P.M. June 2 and incubated at 13° C. At 8 A.M. June 3 ½ of each of 3 leaves was pressed at 50 lb. and the other half left untreated. Six days later 25 lesions were counted on the 3 control halves and 3 lesions on the 3 treated halves. Water soaking of the inoculated leaves at 15 hours after inoculation caused a similar reduction in number of lesions.

Viruses.—Pressing bean leaves immediately before or immediately after inoculation increased the amount of TMV infection, whether or not carborundum or phosphate was used in the inoculation process. The increased infection due to pressing was greatest on leaves which had become somewhat resistant due to age (Figure 3, 4). No effect on infection was observed if the leaves were pressed a day or more before or after inoculation. Inoculation by spraying with a virus suspension without carborundum was successful after pressing the leaf but not without pressing. The presence of water reduces the success of some virus inoculations[3] and water soaking of leaves by spraying them with water before or after virus inoculation decreased the amount of TMV infection. Therefore the pressure effect on TMV infection could not be the result of water soaking of the leaves.

Pressure before inoculation increased the susceptibility of beans to tobacco necrosis virus and tobacco ringspot virus, and of *Nicotiana glutinosa* to TMV, but no effect of pressing on the susceptibility of beans to alfalfa mosaic virus was detected in 1 test.

Various combinations of pressure, carborundum, and phosphate in inoculations with beet curly top virus on bean and beet, and with aster yellows virus on sunflower, did not result in infection.

SUMMARY

Pressing of leaves at 10–240 pounds of pressure per sq. in. killed young infections of bean rust, snapdragon rust, sunflower rust, bean powdery mildew, and spinach downy mildew. The greater the pressure and the younger the leaf the more successful was the therapy and the greater was the leaf injury. Pressing of bean leaves immediately before or after inoculation greatly increased the amount of infection with tobacco mosaic virus, tobacco ringspot virus, and tobacco necrosis virus. Pressing of Pinto bean leaves which had become resistant to bean anthracnose due to age, caused them to become susceptible.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA

[3] Yarwood, C. E. 1952. Deleterious action of water in plant virus inoculations. Nature 169: 502.

A NEW SEROLOGICAL TEST FOR TOBACCO MOSAIC VIRUS[1]

Ellen L. Moorhead and W. C. Price

The precipitin test and the complement fixation test have been widely used in studying serological relationships of plant viruses (1, 2, 7, 8). A quantitative precipitin test requires relatively large amounts of materials (1, 2); the usefulness of the test is thereby limited, especially when purified and concentrated antigen preparations are employed. On the other hand, the complement fixation reaction is laborious and time consuming (7, 8).

This paper describes a simple serological test which is both sensitive and specific for tobacco mosaic virus (TMV) and which has advantages over both the precipitin and complement fixation tests. It uses a minimum quantity of virus solution and antiserum as compared with that needed for the precipitin reaction. Although less sensitive than the complement fixation test, it requires less time and effort. It is also less expensive since the only solutions needed are antigen, antiserum, and fresh sheep red blood cells.

MATERIALS AND METODS.—*Preparation of antigens.*—The viruses used were the Rosette (4) and Type strains of TMV, 7 mutant derivatives of the Type strain, and Southern bean mosaic virus (SBMV). All the strains of TMV were propagated in plants of Turkish type tobacco (*Nicotiana tabacum* L.). SBMV was propagated in plants of the Bountiful variety of garden bean (*Phaseolus vulgaris* L.).

Purified and concentrated preparations of the TMV strains were obtained by differential centrifugation (2 cycles at 2950 g. for 20 minutes separated by a single cycle at 25.000 g. for 2 hours). The pellet obtained by highspeed centrifugation was dissolved in phosphate buffer at pH 7. Identity of the strains present in the purified preparation was checked by subinoculation to appropriate host plants. SBMV preparations were obtained in a similar manner except that 3 cycles of low- and high-speed centrifugation were used.[2]

TMV antigens were stored at −20°C. Aliquots were removed from time to time and kept at 5°C. until used; this ensured a minimum of freezing and thawing. The weight of virus per ml. of solution was determined immediately after preparation by drying a known volume to constant weight in an oven at 105–110°C.

Preparation of antisera.—Healthy rabbits weighing approximately 5 lb. were used for immunization. Normal serum was obtained from each rabbit by cardiac puncture. Rabbits immunized with Type, No. 5, Y14G1, Y14N1Y1, Y20Y1, and Y20R1 strains received 0.1–0.2 ml. of concentrated virus intravenously 2–4 times every other week for at least 6 weeks until it was established that further injections did not increase the antibody level. In the case of the Rosette strain, an attemnt was made to boost the antibody response by a weekly injection of 1.0 ml. of concentrated virus intraperitoneally, in addition to the intravenous injections. Consequently, the total amount of virus injected was 6–20 times that for any other strain, yet the final serum titer was not significantly higher than that for the other strains. This indicates that great amounts of antigen can be wasted in the immunization procedure.

Rabbits immunized with strains 5G1 and Y25 were inoculated by both iv. and ip. routes with clarified infectious sap during 7 weeks when a maximum antibody level was established. They were rested for 8 months, and then received 3 inoculations of purified virus over a 2-week period. As a result of the immunization procedure, the titer of their antiserum was consistently higher than that of any other antiserum.

All animals, except that immunized with the Rosette strain, were exsanguinated at their maximum antibody level to ensure a source of serum which remained constant in titer throughout the tests. The sera were stored at −20°C., aliquots being removed from time to time and held at 5°C. until used.

In preparing antiserum for SBMV approximately 2 ml. of diluted virus (¼) was homogenized with an equal volume of mineral oil (6) and injected into the thigh muscles of a rabbit.

Preparation of sheep red blood cells.—Fresh sheep red blood cells in Alsever's solution were washed 3 times with saline buffered to pH 7.3 with 0.5 M phosphate buffer. After the third washing, the cells were suspended in 5 times their volume of buffered saline, transferred to a 15 ml. conical graduated centrifuge tube, and spun at 2000 r.p.m. for 10 minutes in a clinical type centrifuge. The volume of cells was determined directly, the supernatant fluid removed, and buffered saline added to make a 1 per cent suspension. Successive preparations of red blood cells have approximately uniform density by this method.

EXPERIMENTAL RESULTS.—Lucite plates (5) 7 × 9 × ⅝ in. into which ¾ in. wells had been cut were used in the tests to determine antigenic activity and antiserum titers. The pattern of settled cells could easily be read against a white background. The clear plastic is a convenience but not essential to the test, since it was possible to obtain similar results by reading the extent of settling of cells in Wasserman tubes.

Numerous tests were set up for the antigen-antibody systems used in this study. However, only representative ones will be presented.

Homologous titrations.—Two-fold serial dilutions of concentrated virus and 2-fold serial dilutions of antiserum were prepared in Wasserman tubes using buffered saline (pH 7.3). 0.1 ml. of each dilution of anti-

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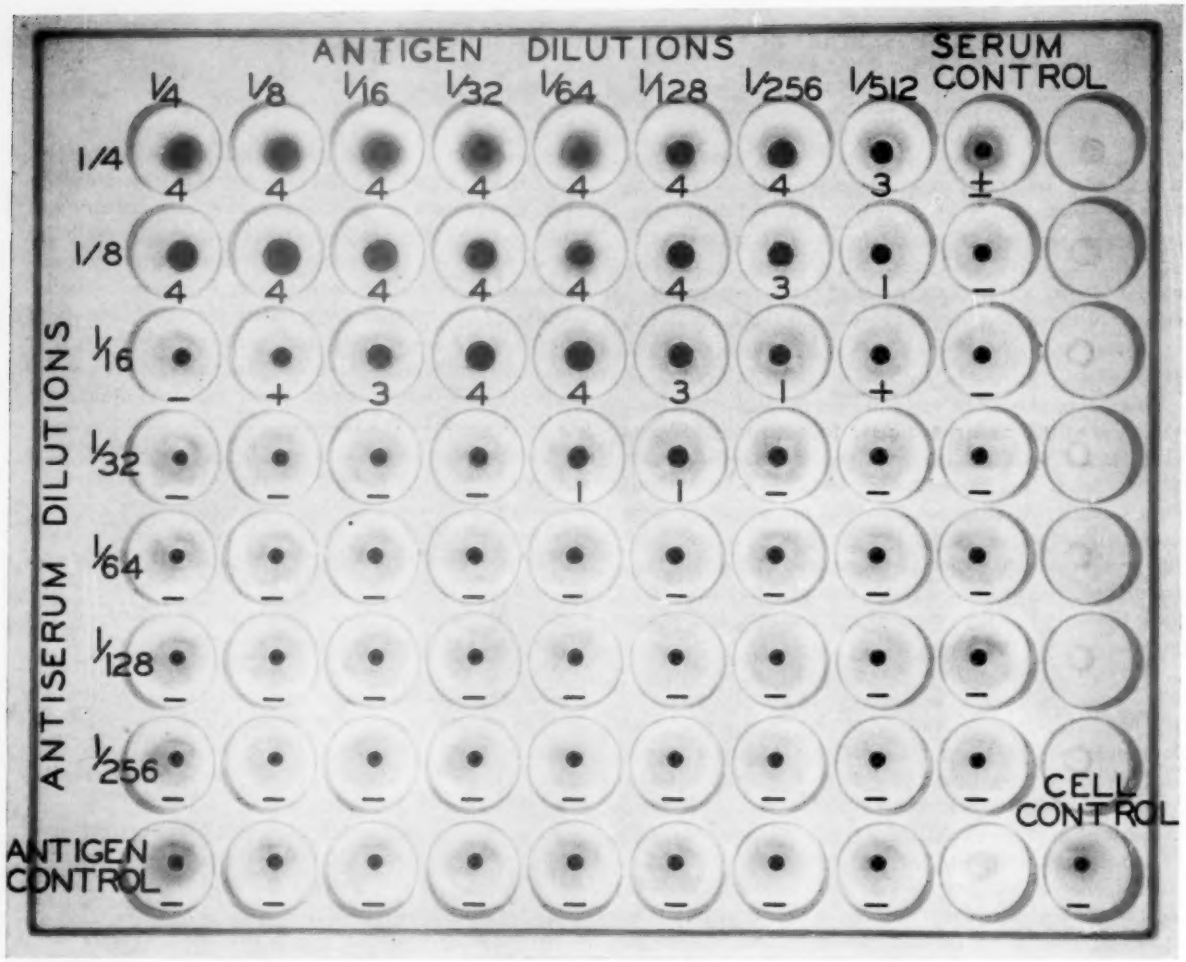
Based on a thesis to be submitted by the senior author in partial fulfillment of the requirements for the Doctor of Philosophy degree.

Contribution from the Plant Virus Laboratory of the University of Pittsburgh.

[2] The authors are indebted to Mr. T. E. Cartwright for the SBMV preparation.


ANTIGEN DILUTIONS
SERUM CONTROL
1/4 1/8 1/16 1/32 1/64 1/128 1/256 1/512
ANTISERUM DILUTIONS
1/4 4 4 4 4 4 4 4 3 ±
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1/64 – – – – – – – – –
1/128 – – – – – – – – –
1/256 – – – – – – – – –
ANTIGEN CONTROL – – – – – – – – –
CELL CONTROL –

Fig. 1. A typical grid titration of TMV with its homologous antiserum by the plastic-plate technique. Each well in the first 7 rows and the first 8 columns contained 0.1 ml. of a virus dilution, 0.1 ml. of an antiserum dilution, and 0.2 ml of a 1 per cent solution of sheep red blood cells; the eighth row contained no antiserum, the ninth column contained no antigen, and the well in the lower right corner contained only sheep red blood cells. The numerals designate the extent of the reaction as determined by degree of settling of the red blood cells. The optimum reacting dilution (ORD) of the antigen was 1/64 while the antiserum dilution endpoint (ADE) was 1/16.

gen and antiserum, respectively, were transferred to the plates. 0.2 ml. of freshly prepared sheep red blood cells were then added to each mixture. The solutions were thoroughly mixed and placed at 5°C. for exactly 3 hours. The pattern of settled cells revealed the extent of antibody-antigen combination. In the absence of antiserum or antigen, and in negative reactions, the sheep red blood cells gradually rolled to the bottom of the well where they appeared as central, sharply defined red discs. In a 4 reaction, indicating maximum combination, the cells settled almost equally over the entire conical bottom of the container with the periphery showing a somewhat heavier precipitate. Intermediate reactions, 3,2,1, and +, could also be observed. The greatest dilution of antigen showing a 4 reaction with the least amount of antiserum was taken as the optimum reacting antigen dilution (ORD) and the highest dilution of antiserum giving a 4 reaction with the least amount of antigen was taken as the antiserum dilution endpoint (ADE) (Fig. 1). The 4 reaction was chosen as a point of reference because this seemed to yield the most consistent and comparable results; similar results could, however, be obtained by choosing a 3,2,1, + or negative reaction as a reference point.

Specificity of the red blood cell test.—To determine whether or not the sheep red blood cell test can be used to detect serological reactions between SBMV and its homologous antiserum and also to determine the degree of specificity of the test for TMV, reciprocal grid titrations were made with the Type strain of TMV, with SBMV, and the respective antisera. Reciprocal grid titrations were made similarly by complement fixation reactions as a control on the technique. Even though both antigens titered to high dilutions (1/4096) in the homologous grids, no cross reaction was obtained in the sheep red blood cell precipitin test. By complement fixation procedures, a greater than 12-fold

drop in antiserum dilution endpoint was observed when the ORD of the heterologous antigen was used. Cross reaction was observed with very low dilutions of antigen (less than 1/64), indicating that TMV and SBMV have minor antigenic properties in common.

Tests with other kinds of red blood cells.—Red blood cells from chicken, mouse, hamster, guinea pig, rabbit, and Rhesus monkey, and human O-type cells were used in TMV precipitin tests similar to those described above. In concentrations of 1 per cent and over a 2-hour period, only weak reactions were obtained with all cells other than sheep erythrocytes. The availability of fresh sheep cells warranted their adoption for this test. It is possible that techniques could be developed using other cells at proper concentrations and pH to give as suitable a test for TMV as that described here.

Effect of concentration of sheep erythrocytes.—Tests were made with 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 5.0 per cent suspensions of sheep red blood cells to determine the best concentration for use in the test. It was found that a concentration of 0.5 per cent was too low to give a clear cut reaction. No significant differences in endpoint titrations among the other concentrations were observed.

Effect of heating antiserum.—Antiserum for the Type strain that had been heated at 56°C. for 0, 5, 15, 20, 30, 60, and 90 minutes was used at a dilution of ¼ in tests with 2-fold serial dilutions of antigen from ¼ to 1/512. No differences among the various samples of antiserum were found. Thus, the presence of complement is not essential for a successful test.

Reactions with normal and infectious plant sap.—Sap expressed from normal healthy tobacco plants gave no reaction with immune rabbit serum. However, both normal and infectious sap caused spontaneous agglutination of sheep red blood cells, as expected (3). The normal agglutinins were removed from sap by the high-speed centrifugation used to concentrate and purify the virus samples.

Heterologous reactions.—Using the ORD of each TMV strain determined from previous homologous titrations, the extent of cross reaction with antiserum for all TMV strains was measured. Data are in Table 1. Antisera for the Type strain and its mutant derivatives reacted with all heterologous antigens, except that of the Rosette strain, at antiserum dilution endpoints that differed from the homologous reaction by not more than 2-fold. Antiserum dilution endpoints in heterologous reactions between Rosette strain antigen and other antisera differed from those obtained in the homologous titrations by more than 2-fold and usually by 8-fold. Thus, differences in antiserum dilution endpoints in homologous and heterologous reactions are capable of distinguishing the Rosette strain from the Type and its mutant derivatives, but not of distinguishing among the closely related mutants.

Similar results were obtained in cross reactions using a constant dilution of antiserum twice as concentrated as the ADE for each strain. The antiserum dilutions chosen were sufficiently concentrated for homologous reactions; they were also sufficiently concentrated for heterologous reactions except with antigen of the Rosette strain. The results thus confirm those obtained in tests for cross reactions between the ORD of each strain and various antiserum dilutions.

TABLE 1.—*Reciprocal of antiserum dilution endpoints with optimum reacting dilutions of all TMV antigens*

TMV Strain Antiserum	TMV strain antigens and ORD								
	Type 32[a]	No. 5 32	Y14G1 32	Y14N1Y1 32	Y20Y1 64	Y20R1 32	5G1 64	Y25 32	Rosette 32
Type	16	16	16	32	16	16	16	32	2
No. 5	16	16	16	16	16	8	8	16	2
Y14G1	16	16	16	16	16	8	8	16	2
Y14N1Y1	16	16	16	16	16	16	8	32	2
Y20Y1	16	16	16	16	16	16	8	32	2
5G1	64	128	32	128	128	64	64	128	16
Y25	64	64	32	64	64	32	32	64	16
Rosette	16	16	8	16	8	16	8	16	16

[a] Reciprocal of antigen dilution.

To determine more completely the range of cross reaction between the Rosette strain antigen and various antisera, reciprocal grid titrations were set up. Homologous antiserum and hyperimmune antiserum of strains Y25 and 5G1 gave endpoint dilutions of 1:16, whereas the remaining heterologous antisera reacted only at a 1:2 dilution. When reciprocal grid titrations were set up with Rosette antiserum and all other TMV strain antigens, no difference in antigen ORD was observed, outside the 2-fold dilution margin of error of the test. The results confirm those of the previous tests in indicating a distinct serological difference between the Rosette strain and the other strains.

Comparison between results with the sheep red blood cell precipitin test and those of the quantitative precipitin reaction.—A quantitative precipitin test with No. 5 strain TMV antigen and antiserum was set up for another purpose. Since the quantitative measure was on the basis of weight of the precipitate, the ordinary test differs in volume, 0.5 ml. of antigen and antiserum, and in incubation time, 48 hours at 5°C, from the sheep red blood cell precipitin test. Results obtained with a grid titration in the quantitative precipitin test were approximately the same as those for the sheep red blood cell test, except that trace reactions were observed in a large number of the higher dilutions of both antigen and antiserum, making detection of a precise endpoint difficult.

Comparison between results of the sheep red blood cell test and the complement fixation reaction.—Homologous grid titrations with all TMV antigens were set up by the complement fixation technique (7,8). The ORD and ADE of each antigen-antibody system were located as previously described. Since the weight of virus in each antigen preparation was known, the

TABLE 2.—*Optimum reacting antigen dilution (ORD), optimum antigenic unit (OAU), and antiserum dilution endpoint (ADE) for all TMV antigens as determined by sheep red blood cell precipitin and complement fixation techniques*

TMV strain	Weight of Virus	Complement fixation			Sheep cell precipitin		
		ORD	OAU	ADE	ORD	OAU	ADE
	mg./ml.	ug./ml.			mg./ml.		
Type	24.3	4096[a]	5.9	128[a]	32[a]	0.76	16[a]
No. 5	21.7	4096	5.3	128	32	0.68	16
Y14G1	18.7	4096	4.6	128	32	0.58	16
Y14N 1Y1	6.3	4096	1.5	128	32	0.20	16
Y20Y1	7.3	8192	0.9	64	64	0.11	16
Y20R1	12.1	4096	3.0	128	32	0.38	8
5G1	26.9	8192	3.3	512	64	0.42	32
Y25	7.5	4096	1.8	256	32	0.23	64
Rosette	31.5	4096	7.7	128	64	0.49	16

[a] Reciprocal of dilution.

amount of reactant in the ORD was calculated. This is the optimum antigenic unit (OAU) and provides another basis of differentiation among TMV strains. For example, it was determined by direct weighing that 1 ml. of No. 5 strain TMV antigen contained 21.7 mg. of virus.[3] Its ORD was 1:32 by the sheep red blood cell precipitin test and 1:4096 by complement fixation methods. Therefore, the OAU for each test is 0.68 mg. and 5.3 μg., respectively. The OAU for each antigen was similarly determined (Table 2).

It has been reported (8) that the OAU determined by complement fixation is capable of differentiating strains of TMV. In exactly the same way the OAU determined by the sheep red blood cell test can be used to differentiate strains of TMV (Table 2). The OAU for the Type strain is 7 times that for strain Y20Y1, which is a highly significant difference. In these tests 2-fold differences in OAU are probably significant; intermediate dilutions within the sensitive range of the antigen-antibody reaction would demonstrate even smaller differences. The data show that the OAU determined by the sheep red blood cell test is approximately 128 times that determined by complement fixation. Thus, while the 2 kinds of tests are equally sensitive in differentiating strains of TMV, the complement fixation test is 128 times as sensitive for detecting presence of virus.

The ratio of the OAU obtained by complement fixation to that obtained by the sheep red blood cell test was constant, the only exception occurring with the Rosette strain when the ratio differed from the others by 2-fold. Thus, the results of the 2 tests are comparable; their accuracy is limited by approximately the same margin of error.

DISCUSSION.—TMV has a long, rod-shaped particle, giving a flocculent type precipitate in combination with its antiserum. The antigen-antibody network formed by this combination is probably quite complex. It is suggested that the sheep red blood cells do not combine in the reaction but merely are caught in the network and thus prevented from settling in a normal manner. Therefore, the cells serve as an indicator of the extent of combination of antigen and antibody in the system. If this explanation is correct then it is not unlikely that similar indicator systems can be found for other plant-virus-antibody combinations.

Advantages of using sheep red blood cells to determine the extent of combination are 1) the amounts of materials used are only 1/5 those required in the quantitative precipitin test, 2) the time required for the various titrations and dilution procedures is of the order of ¼ that necessary for the complement fixation test, and 3) the ease with which the pattern of settled cells is read, especially in the plastic plate technique, makes for greater reliability. Negative reactions can be read with ease, but the various degrees of the positive reaction must be standardized by the individual investigator, as is true also for both complement fixation and precipitin test readings. Too much emphasis can not be placed on the latter point. It is not required that another investigator's results be duplicated quantitatively; with different antigen preparations, other animals, different personal techniques, and variable endpoint readings, such duplication is practically impossible. However, the relative values obtained by various investigations and relationships among them should be comparable.

From evidence presented in this paper it is clear that the sheep red blood cell precipitin test can differentiate strains of TMV 1) by detecting differences in their optimum antigenic units and 2) by detecting differences between heterologous and homologous reactions in antiserum dilution endpoints. The fact that exactly the same sort of differences can be detected by complement fixation provides evidence for identity of the antibodies involved in the 2 reactions. Evidence for non-identity lies in the observation that the ORD in the sheep red blood cell test is 128 times as concentrated as that in the complement fixation test whereas the ADE is only 8 times as concentrated (Table 2).

Since the OAU appears to be a fundamental characteristic of a virus strain it would be possible to titrate an unknown preparation of a given strain of TMV and then to calculate the amount of virus present in the sample, provided that the strength of the antiserum and the OAU for the antigen is first established. The sheep red blood cell test may thus afford a relatively simple method for assaying purified preparations of TMV. It has advantages over the ordinary precipitin test for this purpose, being somewhat more sensitive and requiring somewhat less material. Whether it can be used for assaying for virus in sap from various parts of infected plants will depend upon whether or not the normal agglutinins present in the sap can be absorbed or eliminated in some other manner. This point has not yet been tested experimentally.

[3] Since the OAU for each strain is reproducible with different preparations of purified virus material it is assumed that all, or nearly all, of the material present in the preparation is virus.

A recent trend in the application of the precipitin test to serological research has been the comparison of virus strains with respect to the location of equivalence zones or points of maximum antigen-antibody combination, which is usually just on the antibody excess side of equivalence. Preliminary tests with the supernatant fluids from the sheep red blood cell precipitin test indicated that similar points of reference can be located. Some difficulty may be encountered in determining the proper dilution of antigen for the location of the antibody excess zone since the complex formed is evidently sensitive to excess antigen and probably goes into the soluble state. By testing aliquots of the supernatant fluid with several different antigen concentrations the most appropriate dilution can be found and the difficulty eliminated.

SUMMARY

In a new serological test for tobacco mosaic virus sheep red blood cells were used as an indicator of antigen-antibody combination. Apparently the mode of action of the red blood cells is not one of combination; they seem to become entangled in the antigen-antibody network and are prevented from settling in a normal manner. A way to use the test in assaying purified preparations of TMV is suggested.

Differences among the Type and Rosette strains of TMV and 7 mutant derivatives of the Type strain were detected by means of the sheep red blood cell test; these differences were comparable with those found by complement fixation. Rosette was differentiated from all the other strains by the fact that antiserum dilution endpoints in heterologous reactions between Rosette strain antigen and antisera for the other strains differed from those in the homologous reactions by at least 4-fold, usually 8-fold. Differences as great as 7-fold among strains were found in the weight of virus present in the optimum reacting dilution of antigen, by definition the optimum antigenic unit. Not all strains differed in their optimum antigenic unit. However, the ratio of the optimum antigenic unit determined by the sheep red blood cell precipitin test to that determined by complement fixation was constant, except that the ratio for the Rosette strain differed from the others by 2-fold.

DEPARTMENT OF BIOLOGICAL SCIENCES
UNIVERSITY OF PITTSBURGH
PITTSBURGH 13, PA.

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VARIETAL DIFFERENCES IN SUSCEPTIBILITY OF SWEET POTATO TO BLACK ROT FUNGUS [1]

Pen Ching Cheo

Black rot of sweet potatoes (*Ipomoea batatas* (L.) Lam.), caused by *Endoconidiophora fimbriata* (Ell. & Halst.) Davidson, is one of the most destructive and widely distributed diseases of this crop in the United States. Primarily a seed-bed and field disease, it is also very destructive in storage and transit. While treatment of sweet potato roots with various chemical fungicides and heat have been reported to reduce loss from black rot (1, 3, 4, 5, 6, 7, 8), the development and use of resistant varieties would be a more effective means of controlling this disease.

Few reports have appeared on differences in varietal reaction to black rot, because it has been generally considered that all commercial varieties are susceptible. Harter et al (2) in field tests that included most of the best known commercial varieties, indicated that none of the 21 varieties tested were immune from the black rot fungus. Their judgement was based upon the percentage of infection and the average number of lesions per sweet potato.

Because of lack of knowledge on varietal resistance to the black rot fungus and the prevalent view that no important degree of resistance exists among sweet potatoes in the United States, the following exploratory laboratory and greenhouse tests were made to determine whether or not varietal differences exist.

In the laboratory, 10 varieties were used in each test in comparison with Yellow Jersey, a very susceptible variety. The sweet potatoes were inoculated by introducing a spore suspension of the fungus into the tissue to a depth of approximately 8 mm. with a straight medicine dropper, having a sharp tip, 2 mm. in diameter. Immediately after inoculation, the roots of each variety were placed separately in large covered crystallizing dishes used as moist chambers and held in a controlled room at 70° F. and 90 per cent relative humidity. Measurements of lesions that developed and other observations were made 7, 10, 14, 17 and 21 days after inoculation. Each test was terminated in 21 days, and the infected roots were cut through the point of inoculation for observation of internal reactions to the inoculation procedure and to the black rot fungus.

Results of laboratory tests indicated that, although none of more than 100 varieties tested was entirely immune from black rot infection under these conditions, a few were highly resistant. The varieties tested were classified in 4 categories.

Type I, Highly Resistant—Lesions small, circular, dry and sunken, with clear-cut margins; diameter of lesions not over 1 cm., usually smaller, 21 days after inoculation, (Fig. 1, A). The most active period of lesion enlargement was within 10 days after inoculation after which it was negligible, probably because of formation of a cork layer. Very scanty or no mycelial growth and no sporulation occurred at the inoculation point. Cross-sections of the roots through the point of inoculation showed no penetration of the fungus beyond the stabbed area (Fig. 1, D). A layer of cork cells surrounded the small cavity produced by stabbing the root with the tip of the medicine dropper. Six seedlings that showed this type of resistance are B-5941, B-5944, B-5999, B-6097, K-1716 and Oklahoma 24 (All-gold).

Type II, Resistant—Lesions more or less oval, sunken, and with uneven margins that sometimes showed irregularities due to progress of the fungus beyond breaks and gaps in the cork-layer around the point of inoculation; diameter of the lesions more than 1 cm. 21 days after inoculation. The average rate of increase in size of the lesion remained approximately constant throughout the 21 days. Very scanty mycelial growth and sporulation occurred at the point of inoculation. Cross-sections of roots through the point of inoculation showed very limited fungus penetration into tissue. The cork-layer formation is less effective in arresting growth of the fungus than in roots with Type I resistance. Varieties, seedlings, and introductions showing this type of resistance are: Pierson, B-52, B-5868, B-6271, E-7, Oklahoma 17 and P.I. nos. 153905, 153907, 153909 introduced from Japan.

Type III, Susceptible—Lesions circular to irregular, not sunken, margin indistinct, sometimes surrounded by a water-soaked zone in early stage; lesions large, usually about 2 cm. in diameter 21 days after inoculation, size increasing at an approximately constant rate throughout the period of observation. Abundant mycelial growth and sporulation evident at the point of inoculation (Fig. 1, B). Cross-sections of the roots through the point of inoculation showed the blackened area extending from the small cavity into the surrounding root tissue at an almost equal rate in all directions but somewhat more rapidly in the cortical

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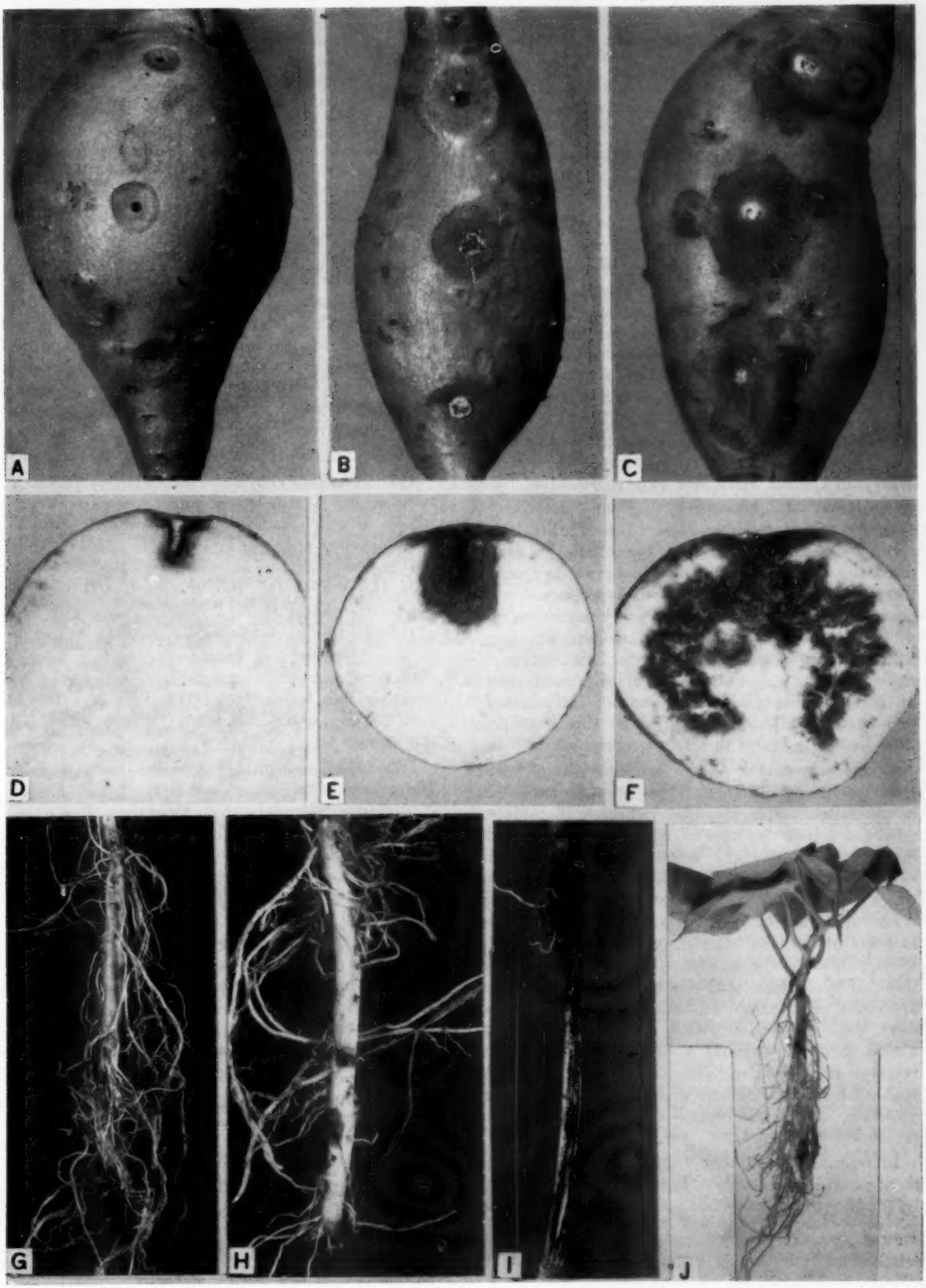
FIG. 1. Varietal differences in reaction of sweet potato to inoculation with the black rot fungus. A) D) Type I reaction, root of All-gold (Oklahoma 24) variety; B) E) Type III reaction, root of Goldrush; C) F) Type IV reaction, root of Orange Little Stem; G) Type I reaction, stem of transplanted draw of seedling B-6097; H) Type II reaction, stem of transplanted draw of seedling K-1716; I) Type IV reaction, stem of transplanted cutting, seedling 10488; J) Type I reaction, transplanted cutting, seedling B-5944.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region than in the deeper tissues (Fig. 1, E). No trace of cork-layer formation was evident. Some susceptible varieties and seedlings are Unit I Porto Rico, Goldrush, Heartogold, Virginian, Southern Queen, Ranger, Nancy Hall, Triumph, Norton, Yellow Strasburg, Australian Canner, Red Nancy, Pelican Processor, Whitestar, Queen Mary, Vates No. 1, Oklahoma 46, Oklahoma 7 and many other seedlings.

Type IV, Very susceptible—Lesions diffused, irregular in shape, not sunken, margins not clear-cut (Fig. 1, C); lesions, large, more than 2.5 cm. in diameter 21 days after inoculation, size increasing at an approximately constant rate throughout the period of observation. Abundant mycelial growth and sporulation occurred at the inoculation point. Cross-sections of the roots through the point of inoculation showed that the penetration of the blackened area into the root tissues was very deep, forming irregular zigzag cavities containing scattered mycelial growth (Fig. 1, F). No trace of cork-layer formation was evident. Some varieties and seedlings showing this extreme susceptibility are Yellow Jersey, Orange Little Stem, Maryland Golden, Rols, Red Jersey, Big Stem Jersey, Orlis, Vineland Bush and many other seedlings.

In the highly resistant class (Type I) the lack of spore production, the limited size of the lesion, and the failure of the rotted areas to extend farther into the root tissue because of the cork-layer formation appear to be of important significance in reducing or avoiding economic losses during storage and transit when this disease is most destructive. Scanty sporulation or lack of sporulation retards or prevents spread of this disease in storage and transit. The small, dry, and superficial lesions occurring in this class cause little loss in the roots that become infected.

Preliminary studies of free hand sections of healthy sweet potatoes stained with 50 per cent alcoholic iodine in potassium iodide solution[2] indicated that there is no difference in the rate of cork periderm formation in the resistant variety B-5941 and the susceptible variety Yellow Jersey. Fresh cuts were made in roots which were then held in moisture chambers at room temperature. Cork periderm formation was evident in roots of both varieties on the fourth day. When slices of roots were sprayed with a spore suspension, those of susceptible varieties and seedlings produced abundant mycelial growth and sporulation within 4 days, while those of resistant varieties produced only a thin layer of mycelial growth and perithecial development was sparse. This indicates a physiologic resistance in the resistant roots. The rate of fungus growth in susceptible roots is faster than the capacity for cork formation.

In repeated greenhouse tests, 25 sweet potato cuttings or sprouts of each variety tested were inoculated by dipping the stems into a spore suspension and then transplanting them immediately in sand in a greenhouse bench. Four days after inoculation, leaves of infected plants were yellow, a characteristic aerial symptom of the disease, and the infected plants began to die. Since sweet potato cuttings and sprouts produce roots very readily, a certain percentage of the plants of some varieties eventually recovered sufficiently to grow more or less normally. This recovery was due to growth of new roots formed near the ground level. Black rot lesions that resulted from inoculations were usually confined to the underground portion of the plants, and seldom if ever extended to the aboveground parts. However, in separate experiments dipping the tops of the plants into the spore suspension and transplanting them in the same way demonstrated that the fungus could infect the leaves and cause brown necrotic local lesions. Following terminal necrotic infection the growing tip and young leaves of susceptible sorts were killed. The plants were removed from the bed as they died, and at the end of a month all surviving plants were removed for examination.

These tests on stems showed 4 types of resistance.

Type 1, Highly resistant—No aerial symptoms. The transplanted stem below ground remains free from infection although a few of the small spreading roots are blackened and killed (Fig. 1, G and J). The following seedlings fall in this class: B-5868 (II),[3] B-5941 (I), B-5944 (I), B-5999 (I), B-6097 (I), 10570 and introduction P.I. No. 190249.

Type II, Resistant—No aerial symptom. Most fibrous roots are infected and the infection extends to the underground part of the transplanted stem causing small, isolated, black lesions (fig. 1, H). Seedlings B-25 (II), B-6198 (III), B-6295, K-1716 (I), and 10561 fall in this group.

Type III, Susceptible—Aerial symptoms evident. The transplanted stems are infected and blackened from the ground level down. More than 50 per cent of the plants may recover following growth of new fibrous roots. In this class are Unit I Porto Rico (III), Heartogold (III) and introduction numbers P.I. 153655 (from Tinian Island), 153905 (II), 153906 (II), 153907 (II), 153909 (II) and many other seedlings.

Type IV, Very susceptible—Aerial symptoms evident. The transplanted stems are infected and blackened from the ground level down (Fig. 1, I). More than 50 per cent of the plants are killed within a month following inoculation. Yellow Jersey (IV), Triumph (III), Whitestar (III), Australian Canner (III) and many other varieties and seedlings are in this group.

Among the varieties listed with Type I resistance less than 10 percent of the plants frequently manifest Type II reactions. High temperatures in the greenhouse favored the recovery of infected plants. Some varieties were tested during the winter at moderate temperature, while others were tested in the spring

[2] Stained for ½ hour, destained with 50 per cent alcohol solution until the starch grains turned blue, and then mounted in Lacto-phenol for observation.

[3] Roman numerals refer to the type of resistance or susceptibility of the roots. The roots of some seedlings were not tested.

and early summer at higher greenhouse temperatures. Small differences between varieties tested in different trials probably have no significance. Differences between the Type III and the Type IV reaction can be detected with certainty only within tests.

Among varieties there is a high correlation between resistance in roots and resistance in stems.

DIVISION OF VEGETABLE CROPS AND DISEASES
PLANT INDUSTRY STATION
BELTSVILLE, MARYLAND

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A ROOT ROT OF SOUTHERN PINE NURSERY SEEDLINGS AND ITS CONTROL BY SOIL FUMIGATION [1]

Berch W. Henry [2]

A perplexing root disease has markedly affected the production of pine seedlings at the U. S. Forest Service, W. W. Ashe Nursery near Brooklyn, Mississippi, since the nursery was established in 1936. The nursery is on previously unbroken forest soil classed as Ruston fine sandy loam with a pH of 4.5 to 5.5. Present studies to develop a control for the disease were initiated in 1947 in cooperation with Region 8 and the Southern Forest Experiment Station of the U. S. Forest Service. Preliminary statements of some of the results reported herein already have appeared (3, 4, 6, 7).

THE DISEASE.—*Hosts.*—Of the 4 pine species grown at the nursery, shortleaf (*Pinus echinata* Mill.), loblolly (*P. taeda* L.) and longleaf (*P. palustris* Mill.) are most seriously affected by the root rot while slash (*P. caribaea* Morel.) is somewhat tolerant. Experimental results presented in this paper are limited to the latter 3 species since only 2 non-replicated trials were made with shortleaf, in which, however, it proved highly susceptible.

Symptoms.—Loblolly seedlings may be killed in large numbers during the summer (Fig. 1, A), but longleaf and slash are seldom killed. However, roots of the 3 species may be damaged severely without noticeable above-ground symptoms. The most significant symptoms appear on the roots of stock[3] when it is lifted for transplanting to the forest.

Affected stock may show symptoms that range from small, red, rough areas on the tap and larger lateral roots, to blackening and roughening of the entire surface of the tap root, which may have few or no laterals, to a rotting away of the lower tap root. Secondary roots frequently form above the rotted part of tap roots (Fig. 1, C). With the exception of the completely rotted roots, only the cortical root tissues appear to be affected. In a relatively mild attack, the diseased cortex often appears to be sloughing off while being replaced by healthy cortex. Lenticels in diseased areas are sometimes enlarged to protruding masses of pink, spongy cells. Areas as small as 32 sq. ft. may contain seedlings in all stages of decline as well as disease-free stock. Diseased seedlings that survive the year in the nursery are usually smaller than healthy seedlings.

Occurrence.—Though the root rot has evidently been present at the Ashe Nursery since its establishment, the severity of disease is highly variable in different areas of the nursery. Of 95 acres available for seed beds, approximately 30 have been used for pine production during the 4 years of experimental studies. Two areas devoted to pine production have been virtually free of disease whereas parts of 3 others have exhibited root rot of severe intensity. In general, the disease has been most severe in low areas of relatively poor drainage.

Effects.—The root rot decreases the stand of seedlings in the seedbed, the percentage of total seedlings that are plantable, and the percentage of seedlings that survive after transplantation to the forest.[4] The

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Acknowledgment is made to Ralph M. Lindgren, Principal Pathologist, Division of Forest Pathology, for invaluable aid and encouragement during the course of these studies.

[2] Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Department of Agriculture, in cooperation with the Southern Forest Experiment Station, New Orleans, La.

[3] Southern pines are usually grown in the nursery for only 1 season. Longleaf is fall-sown (November) or spring-sown (March) while the other species are all spring-sown; all stock is lifted and transplanted to the field the following winter (December-February), being 8-13 months from seed.

[4] Acknowledgment is made to R. M. Allen and E. R. Ferguson of the Southern Forest Experiment Station for supervising the establishment of plantation survival plots and for obtaining much of the survival data.

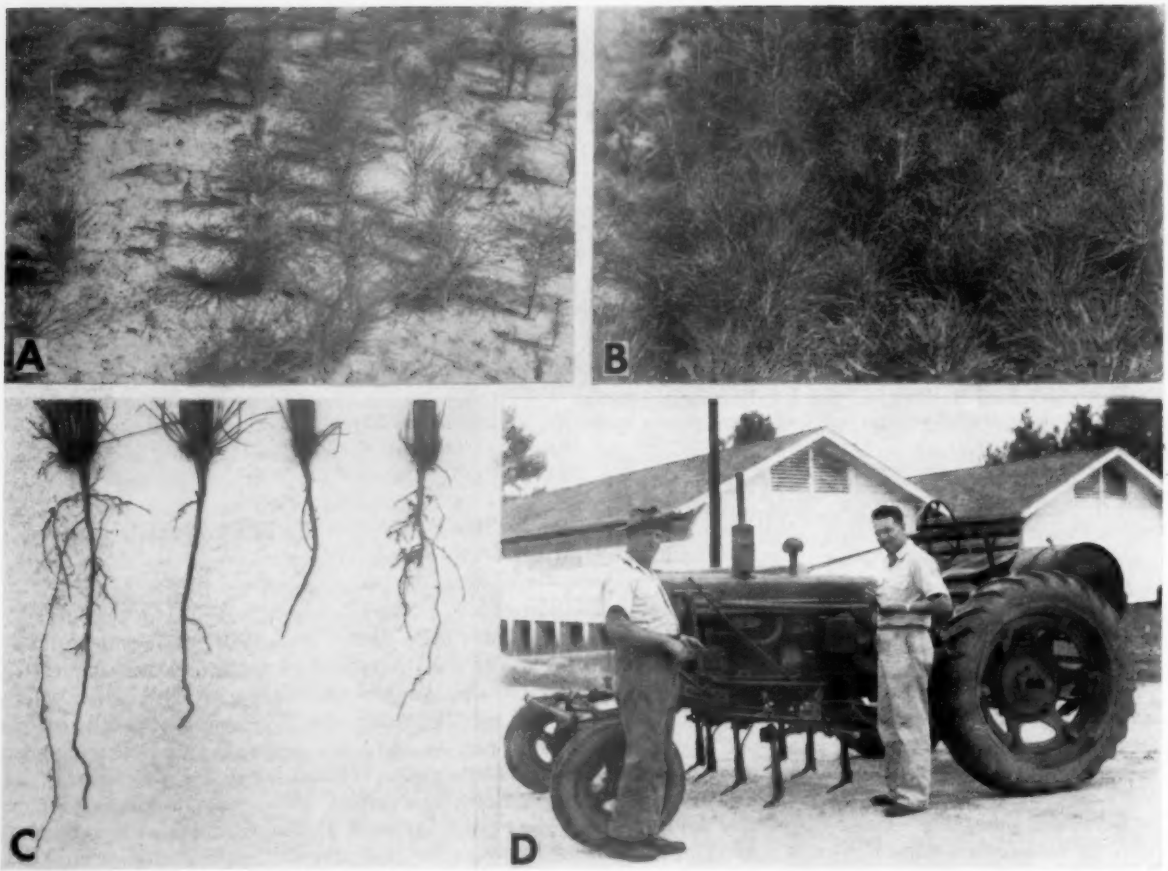

FIG. 1. A and B. Effects of southern pine root rot on 7-month-old loblolly seedlings: A) Untreated; B) Seed bed treated with 32 gallons per acre of 20 per cent by volume solution of ethylene dibromide. C) Root rot on longleaf pine; healthy seedling on left, 3 diseased seedlings on right; lateral roots have formed above dead portion of tap root of seedling on right. D) The Ashe fumigator and the men most responsible for its construction: C. M. Walley, U. S. Forest Service, on left, and H. A. Nation, Dow Chemical Co., on right.

first 2 effects were determined for all experimental nursery plots established during 1947-50. Representative data are in Table 1. These are averages of results of 3 tests with loblolly, 2 with longleaf, and 2 with slash. Each test was of randomized block design with 4 replicates per treatment. Individual plots contained 32 or 200 sq. ft. in any 1 test. The disease-free plots resulted from machine-fumigation with a 20 per cent by volume solution of ethylene dibromide.

TABLE 1.—*Effects of root rot on seedling stand, number and weight of plantable seedlings*

Species	Diseased	Seedlings/sq. ft.		Average green wt./ seedling
		Total	Plantable [a]	
		number	*number*	*gm.*
Loblolly	No	16	15	12
Do	Yes	10	8	12
Longleaf	No	50	41	9
Do	Yes	37	25	8
Slash	No	38	27	9
Do	Yes	36	16	8

[a] Based on U. S. Forest Service standards of size and vigor, irrespective of the presence of root rot.

Fewer seedlings were produced in diseased plots than in disease-free plots (Table 1, Fig. 1, A, B). This reduction in stand was 38 per cent for loblolly, 26 per cent for longleaf, and 5 per cent for slash. Also the percentage of plantable seedlings was lower on diseased plots than on disease-free plots. Seedlings were judged plantable on the basis of U. S. Forest Service standards of size and vigor, irrespective of the presence of root rot. Although loblolly and longleaf suffered greater mortality in the seed bed than slash, a higher percentage of surviving seedlings of loblolly and longleaf met the plantable standards than did slash seedlings. The number of plantable seedlings shows that the total effects of the root rot in the nursery bed is not very different among the 3 species. Thus the reduction in plantable seedlings was 47 per cent for loblolly, 39 for longleaf, and 40 for slash. This reduction is believed to be entirely due to root rot, since the fumigant has shown no beneficial results in seedling production other than disease control.

TABLE 2.—*Field survival of transplanted seedlings*[a] *in respect to severity of root rot*

Species	Months after transplanting	Healthy	Disase class [b]		
			1	2	3
		per cent	*per cent*	*per cent*	*per cent*
Longleaf	6	89	82	70	59
	12	77	52	41	28
	24	74	44	25	25
Slash	6	93	97	96	91
	12	84	90	91	77
	24	74	80	77	68

[a] All transplanted seedlings were from untreated nursery beds and were plantable according to U. S. Forest Service standards. The outplanting was of randomized-block design with 25 seedlings per row and 8 rows per root-rot class.

[b] Class 1 indicates 1 to 25 per cent of tap root surface affected; class 2, 26–50 per cent; class 3, over 50 per cent.

The effect of the disease on survival of seedlings transplanted to the forest is shown in Table 2. The disease had a markedly adverse effect on the survival of longleaf seedlings in the outplanting, both in the relatively lightly affected seedlings of class 1 and the more severely diseased seedlings of classes 2 and 3, which gave the poorest survival. Survival of the diseased longleaf seedlings after 6 months in the field was not especially indicative of their ultimate survival; after 24 months, survival of healthy seedlings was 83 per cent of that at 6 months while of the diseased classes it was 54, 36, and 42 per cent respectively. In contrast to its effect on longleaf, the root rot did not adversely affect the field survival of slash pine. Diseased slash seedlings that reached the plantable category survived as well as healthy ones. Also, the reduction in survival between 6 and 24 months in the field was about the same for the disease-free and the 3 disease classes; the 24 months survival was 80, 82, 80, and 75 per cent, respectively, of that at 6 months. A similar test with loblolly pine, for which 12 months data are available, indicates that this species is similar to slash in that survival of plantable seedlings was not adversely affected by root rot.

Cause.—Sleeth[5] demonstrated that the disease is infectious by reproducing it on seedlings grown in sterilized soil inoculated with bits of diseased roots, and by eliminating it from naturally infected soil by saturation with 5 per cent formaldehyde. Various fungi, including *Fusarium* sp., *Torula marginata* Jackson, and *Sclerotium bataticola* Taub., were isolated from diseased roots by Lamb, Davis, Sleeth and others[6]. However, pathogenicity was not satisfactorily established in any case. Lamb discovered nematodes in diseased roots in 1939[7]. Dr. G. Steiner found nematodes in diseased root specimens submitted by Lamb and identified *Acrobeloides* sp., *Aphelenchoides parietinus* Bastian, and *Panagrolaimus subelongatus* Cobb, none of which he considered responsible for the disease.

In the present study, the 3 above-named fungi, plus *Penicillium spp.*, *Cephalosporium sp.*, an unidentified sterile fungus, and bacteria, have often been isolated from diseased roots but not consistently so, and again pathogenicity was not established in any case. Isolation of *Phytophthora cinnamomi* Rands was attempted from numerous samples of nursery soil, using the apple technique. The results were negative. Dr. W. A. Campbell, Division of Forest Pathology, kindly performed this test. In addition to the 3 above-named nematodes, the following have also been found associated with pine roots in the nursery and were identified by Dr. Steiner: *Diplogaster sp.*, *Discolaimus sp.*, *Ditylenchus sp.*, *Dorylaimus sp.*, *Pratylenchus leiocephalus* Steiner, *Tylenchus filiformis* Bütschli, and *Zeldia* sp. In limited trials, seedlings growing in sterilized soil inoculated with species of nematodes or combinations of species with and without the above-named fungi, failed to develop typical symptoms of the disease.

CONTROL.—*Methods and Materials.*—All tests of methods of controlling the disease were conducted in the nursery. Regular nursery practices were followed insofar as possible. The same seed lot, and rate and date of sowing were used in any 1 experiment. A randomized block design with 4 replicates per treatment was used unless stated otherwise. Individual plots were 4 × 8 ft. or larger, as stated. The shorter plots were separated by 6-in. wide strips of asphalt roofing inserted vertically prior to treating.

Data were obtained from representative seedlings at the end of the growing season. Sufficient seedlings were lifted from each plot, exclusive of a 6-in. border, to furnish the required number of samples of plantable seedlings for root rot and weight determinations, and for field outplantings. Each sample contained a minimum of 25 seedlings. The seedlings from each individual plot were manually randomized before they were graded and samples selected.

Root rot severity of plantable seedlings was determined after individual grading on a 0–100 scale; seedlings rated zero were free from root rot and those rated 100 had the tap root entirely affected and the lower part killed. A severity index of 10 or less indicates no significant damage.

As previously reported (6), the soil fumigants ethylene dibromide and chloropicrin provided control of the disease in limited trials in 1947 while none of the nonfumigant fungicides used was effective.

During the ensuing 3 years, the following materials were applied as soil fumigants prior to seeding the nursery beds: ethylene dibromide as Dowfume W-40, Dowfume W-85 (both from Dow Chemical Co.) and Terrafume-40 (Thompson-Hayward Chemical Co.); chloropicrin as Larvacide (Innis Speiden Co.); methyl bromide gas as MC-2 (Dow Chemical Co.); allyl

[5] Unpublished report in Division of Forest Pathology files, dated Feb. 18, 1941.

[6] Unpublished reports in Division of Forest Pathology files, 1936-1941.

[7] Letter to Dr. G. Steiner, Division of Nematology.

TABLE 3.—*Effects of soil fumigants on seedling production, root rot occurrence, and field survival* [a]

Treatment			Seedlings/sq. ft.		Average green wt./ seedling	Root Rot		Field survival at 12 months
Material	Rate/ acre	Pine Species	Total	Plantable		Seedlings diseased	Severity index [b]	
	gallons		*number*	*number*	*grams*	*per cent*		*per cent*
		longleaf	15	11	8.4	15	4	73
Dowfume W-40	32	slash	46	32	5.9	0	0	79
		loblolly	29	25	9.6	33	9	83
		longleaf	28	24	10.9	6	2	76
Larvacide	41	slash	44	29	7.7	55	24	66
		loblolly	32	27	9.0	68	33	73
		longleaf	16	10	9.2	95	59	46
Dowfume N	50	slash	46	18	6.2	96	47	42
		longleaf	14	12	8.6	75	30	59
Allyl bromide	25	slash	41	27	6.2	63	20	76
		longleaf	20	15	10.5	54	22	59
Dichlorobutene	50	slash	44	26	6.6	75	33	69
		longleaf	21	12	10.5	97	69	34
Untreated		slash	44	16	5.6	95	57	67
		loblolly	15	13	10.5	95	76	83

[a] Only seedlings plantable on a size and vigor basis were considered, except in "Seedlings/sq. ft., Total" column.
[b] Based on a scale of 0 (none) to 100 (severe).

bromide (Dow Chemical Co.); dichloropropane-dichloropropene as Dowfume N (Dow Chemical Co.); dichlorobutene (E. I. DuPont de Nemours and Co.); and chlorobromopropene (Shell Chemical Co.).

With the exception of methyl bromide gas, the fumigants were applied as liquids with the aid of commercial hand applicators or a tractor-mounted apparatus. Applications were made in the tilled seedbed at a depth of 6 in. with 10-in. spacing between injections. The tractor-mounted apparatus, constructed at the nursery, delivered the chemical in a constant stream under pressure (Fig. 1, D). The recommendations of Christie (1) were followed in general. From 2 to 3 weeks elapsed between application of the fumigants and seeding with pine.

The methyl bromide gas was applied under a gas-proof paper cover as directed by the manufacturer[8].

Results with various fumigants.—Representative results with some of the liquid fumigants on seedling production, disease control and survival of transplants are in Table 3. The fumigants were applied with hand applicators. The field test of survival was made with 4 replicates of 25 seedlings each in a randomized block pattern. Each fumigant reduced the severity of root rot and all except Dowfume N reduced the amount. However, none but Dowfume W-40 consistently held the severity index below 10, which was the selected maximum value for practical control. Although this was the only form of ethylene dibromide used in this experiment, a later experiment, with loblolly pine, indicated that the 3 formulations of ethylene dibromide were about equally effective. The relative degree of root rot control by the various fumigants was reflected to some extent in the number of plantable seedlings produced. The exception was longleaf treated with W-40. However, there was always considerable mortality of longleaf following application of W-40 by means of hand applicators. This never occurred following application of the material with the tractor-mounted apparatus (Table 1; see also data on fall and spring fumigations). With slash and loblolly about 100 per cent more plantable seedlings were produced on the W-40 treated plots than on untreated plots. Larvacide was equally effective in producing plantable stock but less so in controlling root rot.

Survival of seedlings, 12 months after transplanting to the field, again showed that badly diseased longleaf nursery stock survived poorly and that the amount of disease had little effect on the survival of slash and loblolly (Table 3). There was little evidence that the fumigants themselves had any effect on subsequent field survival, with the exception of Dowfume N on slash pine. In this case, stock from treated nursery plots survived less successfully than that from untreated plots even though it was less severely diseased.

As a further test of possible adverse effects of Larvacide and Dowfume W-40 on seedling production and subsequent field survival, treatments of 41 and 32 gal. per acre, respectively, were made in a disease-free area of the nursery and plots were sown to longleaf, slash, and loblolly pine. The quantity and quality of seedlings produced were not affected by treatment except that a poor stand of longleaf resulted from applying W-40 with a hand gun, as mentioned earlier. Seedlings from each treatment were transplanted to the field in a randomized block experiment with 8 rows of 25 seedlings each per treatment for longleaf and slash and 5 rows for loblolly. After 30 months, the percentage of survival for the longleaf, slash, and

[8] Dowfume MC-2 for soil treatment. Technical Service and Development, Dow Chemical Company, Midland, Mich., Nov. 1, 1948 (mimeographed).

loblolly pine, respectively, were: Larvacide-treated, 70, 74, and 82; W-40 treated, 72, 80, and 92; untreated, 68, 72, and 85. Thus, in the absence of root rot, field survival was not adversely affected by Larvacide or Dowfume W-40, but instead appeared to be enhanced by the latter. In respect to treatments, there were no differences in the height of seedlings after 30 months in the field.

In later experiments, 2 additional fumigants were included in the comparisons.

Chlorobromopropene was applied at the rate of 32 gal. per acre in an experiment with loblolly pine and gave a fair degree of root rot control; 52 per cent of the plantable seedlings were diseased and the severity index was 24, while 95 per cent of seedlings from untreated plots were diseased and the severity index was 65. In the same experiment, only 7 per cent of the seedlings from plots treated with 32 gal. per acre of Dowfume W-40 were diseased and the severity index was only 2.

Methyl bromide (MC-2) was used at the rate of ½, 1, and 2 lb. per 100 sq. ft. in a duplicate-plot experiment with longleaf and slash pine. The plots were each 4 × 25 ft. and were seeded 24–72 hours after treatment. The disease severity ratings of seedlings from plots treated with the 3 rates respectively were: for longleaf 28, 2, and 0, with 52 for untreated; for slash 22, 0, and 0, with 45 for untreated. Thus the 2 higher rates of the chemical gave excellent disease control. In all treated plots, excellent weed control resulted. However, there was considerable stunting of slash seedlings and fewer reached plantable size in the treated plots than in the untreated, although the total number of seedlings was not reduced.

Of the fumigants tested, ethylene dibromide and methyl bromide gas gave excellent root rot control. Since ethylene dibromide was superior to the other liquid fumigants in controlling the disease and seemed to have no adverse effects, when applied by machine, and since methyl bromide gas was unsatisfactory because of its hazardous nature, the stunting effect on slash pine, and the high material and labor costs involved, further experiments aimed at developing a practical control procedure were limited to the use of ethylene dibromide (EDB).

Residual carry-over effects of EDB.—The possible carry-over of disease control to successive pine crops following fumigation with EDB and the possible phytotoxic effects of repeated treatments were investigated.

Loblolly pine was sown in 1950 on plots that had produced pine seedlings for each of the 2 preceding years and had been fumigated with 32 gal. per acre of a 20 per cent by volume solution of EDB (Table 4). The fumigant was applied in 1948 and 1949 with a tractor-drawn apparatus furnished by the Dow Chemical Company, and in 1950 by the tractor-mounted apparatus built at the nursery. The data represent averages from triplicate plots, of 4 × 25 ft. each, for each treatment.

It was evident that the disease-controlling effects of EDB were still present, to a lesser degree, in the second successive pine crop following fumigation (1949 treatment, Table 4) but virtually absent in the third successive crop (1948 treatment, Table 4). In other experiments, the carry-over of disease control to the second crop of slash and longleaf seedlings was in general less than that shown for loblolly. The disease severity index for loblolly produced as the second crop following fumigation was 28, or considerably higher than the selected maximum of 10 for satisfactory control. The common cultural practice is to have 1 or 2 green manurial crops between pine crops, in which case the residual effectiveness of a fumigant on control of disease on subsequent pine crops would ex-

TABLE 4.—*Carry-over of disease control and effects of repeated seed bed treatments with ethylene dibromide on loblolly pine seedlings produced in 1950*

When Treated	Seedlings/sq. ft.		Average green wt./ plantable seedling [a]	Root rot in plantable stock	
	Total	Plantable		Seedlings diseased	Severity index[a]
	number	*number*	*grams*	*per cent*	
Never	6	5	13	99	77
1948	7	6	11	99	81
1949	9	9	14	58	28
1948 and 1949	9	9	12	44	20
1950	9	9	13	29	8
1948 and 1950	11	11	13	18	5
1949 and 1950	11	10	12	26	7
1948, 1949 and 1950	10	10	14	6	1

[a] Based on a scale of 0 (none) to 100 (severe).

TABLE 5.—*Relative effectiveness of fall versus spring fumigation with ethylene dibromide in controlling root rot*

Pine species and season sown	Season treated	Seedlings/sq. ft.		Average green wt./ plantable seedlings	Root rot on plantable stock	
		Total	Plantable		Seedlings diseased	Severity index [a]
		number	*number*	*grams*	*per cent*	
Longleaf, fall 1949	Fall 1949	31	31	11	0	0
	Never	30	30	11	0	0
Longleaf, spring 1950	Spring 1950	39	38	5	4	1
	Fall 1949	44	43	6	41	12
	Never	30	28	5	72	42
Loblolly, spring 1950	Spring 1950	----	----	----	4	1
	Fall 1949	----	----	----	35	14
	Never	----	----	----	60	36

[a] Based on a scale of 0 (none) to 100 (severe).

pectedly be further reduced. Thus it appeared advisable to recommend fumigation prior to each spring sowing of pine.

Successive annual treatments with EDB produced no adverse effects on the loblolly seedlings and showed some cumulative effects on disease control (Table 4).

Fall vs. spring fumigation as nursery practice.—Because the seasonal work load in the nursery is likely to be heaviest in the spring, it would be advantageous to incorporate the additional practice of soil fumigation into the fall work schedule. Consequently, an experiment was conducted to determine the relative effectiveness in controlling root rot of fall versus spring fumigation with EDB.

Three adjacent nursery beds, 4 × 400 ft. each, were used in the experiment. In 1 bed, alternate 50-ft. plots were machine treated with 30 gal. per acre of a 20 per cent by volume solution of EDB on October 26, 1949. The remaining plots were untreated. The bed was sown to longleaf pine on November 15. In the second bed, two 66-foot plots were fumigated on the same data as above, 2 were fumigated March 12, 1950, and 2 remained untreated. This bed was sown to longleaf pine on March 29. The third bed was treated like the second but was sown to loblolly pine on April 14. Seedlings from all beds were lifted in December 1950. Results are in Table 5.

The lack of root rot on fall-sown longleaf pine seedlings has been reported previously (7) and was confirmed in this experiment.

The fall treatment with EDB resulted in a degree of root rot control sufficiently inferior to the spring treatment to demonstrate that the latter was definitely preferable for spring-sown crops.

Soil conditions in relation to fumigation.—In reports of soil fumigation as a method of disease control, the importance of the condition of the soil at the time of and immediately following fumigation has been repeatedly mentioned (1, 8, 10). The soil type, texture, temperature, and moisture have been considered to be important. In the present work, only the most practical aspects of these factors have been considered.

The soil type could not be altered appreciably in a short time. Since tilling the soil to the extent of acquiring seed-bed quality prior to fumigation entailed no additional work, this was consistently done. At the time when the fumigation procedure fell naturally into the nursery schedule, soil temperatures at a 6-in. depth were within the range recommended (1). EDB provided excellent root rot control when applied at soil temperatures from 55°–72° F. The moisture content of the soil was naturally variable because of heavy spring rains. It could also be made more moist as desired by use of the overhead irrigation system. Consequently an experiment was performed to ascertain the effects of different soil moistures at the time of fumigation on root rot control.

The experimental plots were in a randomized block design with each treatment in triplicate. The soil was in good tilth and its temperature 55° F. at a depth of 6 in. To the 6 plots, approximately 7 qt. of water per sq. yd. were applied with sprinkling cans. This made them wet but not muddy. After a few hours, these plots and 6 others were treated with a 20 per cent by volume solution of EDB at the rate of 32 gal. per acre. Six additional plots remained untreated. Three weeks later, 3 plots of each treatment were sown to longleaf pine and 3 to slash pine. The root-rot severity indices for the resulting seedlings were: longleaf fumigated wet, 2; longleaf fumigated not wet, 1; longleaf not fumigated, 63; slash fumigated wet, 4; slash fumigated not wet, 3; and slash not fumigated, 56. Thus

ethylene dibromide controlled the root rot equally well when applied to very wet soil and to soil in good tilth.

Rate of application of EDB.—After it was demonstrated that spring application of 32 gal. per acre of a 20 per cent solution of EDB effectively controlled the root rot, 2 experiments were conducted to determine the lowest practical rate that would give adequate control. In the first, rates of 24, 32, and 40 gal. per acre were applied with a hand applicator, and the 3 species, longleaf, slash, and loblolly pine, were used. The root rot severity indices of seedlings produced on plots receiving the 3 rates and no treatment respectively were: longleaf, 17, 4, 4, and 69; slash, 3, 0, 6, and 57; loblolly, 9, 9, 9, and 76. In the second experiment, the individual plots were 4 × 50 ft.; rates of 16, 24, and 32 gal. per acre were applied with the tractor-mounted apparatus. Loblolly pine was used. The root rot severity indices for the 3 rates and untreated control, respectively, were: 8, 7, 4, and 82. On the basis of these data, a rate of 24 gal. per acre was expected to provide root rot control with an adequate margin of safety.

Large-scale application of EDB.—As evidence developed that the practical control of root rot was feasible through the use of ethylene dibromide, experiments aimed at the successful treatment of large areas were conducted concurrently with some of those reported above. Hand applicators are inefficient where more than a few hundred sq. ft. must be treated at a time.

In 1948 and 1949, a few nursery beds were treated with a tractor-drawn apparatus furnished by the Dow Chemical Company. Results were excellent and it seemed worthwhile to construct an apparatus to be used on one of the nursery tractors. This was done by U. S. Forest Service nursery personnel with technical assistance from the Dow Chemical Company.

The fumigation apparatus, which became known as the Ashe Fumigator, is shown mounted on a Farmall H. tractor in figure 1, D. A pump, attached to the power-take off of the tractor, delivers the chemical from a 50-gal. drum back of the driver's seat to a boom attached to the hydraulic lift under the tractor. Two rows of shanks, 3 and 4 per row with a lateral spacing of 10 in. between shanks, are also attached to the lift. Down the back of each shank is a metal tube with a nozzle just behind the point of the shank. The chemical is carried by individual hoses from the boom to the tube of each shank. The shanks are inserted 6 in. into the soil when the machine is operating. The rate of delivery is governed by adjusting a valve on the bypass pipe leading back into the drum. The rate of application is governed by the rate of delivery and the operating speed of the tractor. A small cultipacker is attached to the rear of the tractor to seal the furrows left by the shanks. The apparatus can be operated at approximately 4.7 mi. per hour and at that speed a 6- × 415-foot nursery bed can be treated in approximately 1 minute.

The Ashe Fumigator was first used in the fall of 1949. About 15 acres of the nursery were fumigated and then lay fallow until the spring pine crop was sown. The resulting root rot control in areas of high disease severity was of the same magnitude as that previously reported for fall fumigation. About 20 acres were treated in the spring of 1951 and sown to pine. Root rot control was excellent in the resulting crop and no culling or grading was necessary because of the disease. Representative data of root rot control as a result of machine treatment are in Tables 1, 4, and 5 and elsewhere in this paper. The total cost of labor and materials for fumigating with EDB at the recommended rate has been less than 50 dollars per acre. Thus the production costs are increased only a few cents per thousand seedlings.

Recommendations for control of the root rot of pine seedlings at the Ashe Nursery, by soil fumigation with EDB, have been outlined by Maki and Henry (7).

DISCUSSION.—The root disease of pine seedlings described here is believed to be similar to root diseases of tobacco, corn, small grains and other crops as reported by several authors (2, 5, 9, 11, 12). They have much in common in symptoms, association with nematodes, and methods of control. The importance of such root rot disease-complexes has become increasingly evident in recent years. The fact that many of them can be economically controlled by soil fumigation will undoubtedly result in increased study and use of this means of combatting disease.

Though ethylene dibromide has been recommended for controlling the root rot of pine seedlings at the Ashe Nursery, the author does not mean to imply that it is the only material capable of doing so. Considering the 4 factors—disease control, lack of phytotoxicity, safeness of handling, and cost of material—ethylene dibromide proved superior to the other materials tested. Its apparent phytotoxicity to longleaf seedlings when applied with a hand applicator has not been satisfactorily explained. However, since no such adverse effect resulted from machine application, it has not been considered of practical significance in this control program.

Studies on this root rot of pine seedlings have resulted in one of those paradoxical situations where the means for control of a disease have been developed without complete knowledge of its cause. Though circumstantial evidence strongly indicates a nematode-fungus complex, complete proof is still lacking. Similar root disorders are known to exist in other nurseries and on other forest-tree species. Without knowledge of the cause, however, it is understandably difficult to determine if these disorders are one and the same disease. For the same reason, one cannot recommend to other nurseries the control measures described herein except on an experimental basis.

SUMMARY

Symptoms of a root rot of *Pinus palustris, P. caribaea, P. taeda* and *P. echinata* at the U. S. Forest Service's W. W. Ashe Nursery, Brooklyn, Mississippi are

described.

It caused mortality in the seed bed, decreased production of plantable seedlings, and lowered survival of seedlings after transplanting to the forest.

The cause of the root rot has not been clearly demonstrated. Circumstantial evidence indicates a nematode-fungus complex.

Several soil fumigants, applied to the nursery beds prior to seeding, gave varying degrees of control. Considering disease control, phytotoxicity, safety to personnel, and cost of material, ethylene dibromide was superior to all others tested.

The application of 24 gal. per acre of a 20 per cent by volume solution of ethylene dibromide 2–3 weeks prior to spring seeding afforded excellent root rot control with no phytotoxic effects and at a cost of less than 50 dollars per acre.

There was appreciable residual disease-controlling effect of ethylene dibromide to the second successive pine crop, but it was not of sufficient magnitude to eliminate the need for repeated treatment. There were no deleterious effects on the pine crops produced following 2 and 3 successive annual treatments with ethylene dibromide.

Fall fumigation with ethylene dibromide followed by spring sowing of pine, was considerably less effective in controlling the root rot than was spring fumigation.

Ethylene dibromide was equally effective when applied to soil in good tilth and to wet soil.

A tractor-mounted fumigation apparatus, constructed by U. S. Forest Service and Dow Chemical Company personnel, was capable of treating a 6- × 415-ft. nursery bed with ethylene dibromide in approximately 1 minute.

Seed bed fumigation is now incorporated as regular procedure for spring-sown pine at the W. W. Ashe Nursery.

HARRISON EXPERIMENTAL FOREST
SAUCIER, MISS.

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A COMPARISON OF TWO METHODS OF PREPARING TOBACCO MOSAIC VIRUS FOR SPECTROPHOTOMETRIC ASSAY [1]

David E. Schlegel and T. E. Rawlins

The spectrophotometric method as used by Takahashi[2, 3], has been found to be among the most accurate technics for determination of tobacco mosaic virus concentration. In this contribution we compare the results obtained by means of Takahashi's "2-ultra" spectrophotometric method with those obtained by means of a recently developed "heat-ultra" method; the latter involves heating the homogenate to aggregate impurities before ultra-centrifugation of the virus. This treatment apparently recovers a more constant proportion of the virus in the tissue at different virus concentrations than does the 2-ultra method; it also gives less variation in virus recovery from replicate samples taken from a particular virus homogenate.

METHODS.—The 2-ultra method was essentially that used by Takahashi[3] and involves a preliminary low speed centrifugation to sediment the coarser material, followed by 2 cycles of alternate high and low speed centrifugation to purify the virus. The virus concentration was then determined by reading the optical density with light of 260 mμ wave length on a Beckman spectrophotometer.

The heat-ultra method has not been described previously and will therefore be discussed in detail. Lateral mature leaves (Turkish tobacco var. Trelease) inoculated with tobacco mosaic virus (TMV) were used. The youngest and oldest leaves were discarded, and discs 14 mm. in diameter were punched from the infected leaves by means of a sharp cork borer. The first disc was placed in a vial containing Sample 1, the next adjacent disc in a vial containing Sample 2, etc., until there were 20 discs in each sample. This procedure provided the desired number of samples, each weighing about 0.5 gm., and each containing essentially the same amount of virus. To study the effect of a chemical compound on virus multiplication the discs were floated on solutions of the compound while virus multiplication was in progress. The vials containing the infected discs were stoppered, and the samples were frozen at −18° C. Each sample was then thoroughly homogenized in a conical ground glass homogenizer by means of a motor-operated lucite pestle. This homogenization required about 2 minutes. During the grinding just enough distilled water was added to maintain a rather fluid paste conducive to rapid homogenization. When the sample was thoroughly homogenized, it was diluted with more water and poured into a glass stoppered 5 ml. volumetric flask; the homogenizer and pestle were washed several times with enough distilled water to bring the flask to volume. The stoppered flask was shaken to produce thorough mixing, and a 3 ml. aliquot pipetted into a 11 × 75 mm. glass centrifuge tube. The tube was placed in a water bath at 60° C. for 15 minutes to coagulate host proteins, a modification of the method of Steere and Williams.[4] The centrifuge tube containing the sample was centrifuged at 2,000 × gravity for 30 minutes, and the supernatant poured immediately into a small beaker. A 2 ml. aliquot of this sample was pipetted into a 13 × 28 mm. plastic ultracentrifuge tube and centrifuged in a small air driven ultracentrifuge at 75,000 × gravity for 1 hour to sediment the virus. The supernatant was discarded immediately by inverting the centrifuge head. The tubes were removed from the inverted head and drained on filter paper for about 10 minutes. The inside wall near the mouth of the inverted tube was wiped with clean filter paper to remove adhering supernatant fluid. About 1 ml. of 0.01 molar phosphate buffer (pH 7.0) was added to the virus pellet in the tube and allowed to stand overnight at 1–10° C. to hydrate the virus pellet. It was then shaken on a shaking machine for ½ hour to suspend the virus in the buffer. The buffer containing the virus was pipetted from the tube and placed in a 3 ml. volumetric flask. The centrifuge tube was washed several times with buffer, and the washings pipetted into the flask to bring it to volume. The contents of the flask were mixed and then poured into a 11 × 75 mm. centrifuge tube in which they were centrifuged at 2,000 × gravity for 30 minutes. The virus concentration of the supernatant was assayed by determining the optical density with a wave length of 260 mμ in a Beckman spectrophotometer, and estimating virus concentration from the absorbance-virus curve of Takahashi[2].

Experimental.—Since preliminary experiments indicated that the heat-ultra method gives good results, it was considered advisable to determine the best temperature and time of heating for use in the method. The following experiments were therefore conducted: 6 grams of leaf tissue from plants infected for 6 days were homogenized and diluted to 60 ml. with distilled water. Three 3 ml. aliquots of this virus suspension were heated for 15 minutes at each of the following temperatures: 50°, 55°, 60°, 65°, 70°, and 75° C. The remainder of the heat-ultra method was applied to each and the recovered virus assayed. All temperatures tested gave essentially the same virus recovery. Sixty degrees centigrade was therefore chosen as the temperature to be used because it was near the middle of the range tested and gave reproducible results.

The same technic was used to determine the effect

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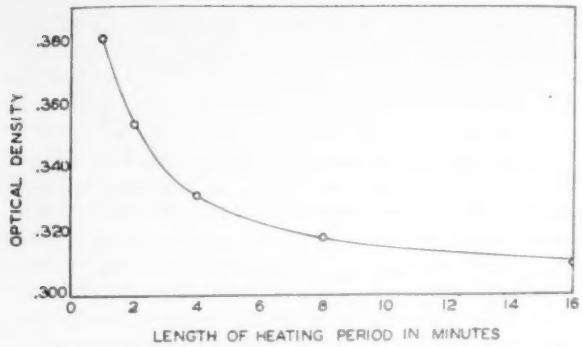

FIG. 1. The virus yield (optical density) in relation to the length of the heating period, showing that slight variations in heating periods longer than 10 minutes result in a minimum variation in virus yield.

of the time of heating on virus recovery during the heat-ultra method. Three 3-ml. aliquots were heated at 60° C. for each of the following periods: 1, 2, 4, 8, and 16 minutes. Average values for the optical density for each period were plotted against the length of the heating period in Figure 1. A heating period of 1 minute gave the highest recovery but here a slight variation in length of the heating period caused a relatively large change in virus recovery. A heating period of 15 minutes was chosen because at this period a slight variation in the time of heating caused a relatively small change in the amount of virus recovered.

The 2-ultra and heat-ultra methods were compared with respect to ability to recover virus from homogenates containing known dilutions of an original virus homogenate. In 1 series of experiments a given fresh weight of healthy and mosaic leaves was homogenized separately; each was diluted with the same amount of 0.1 molar neutral phosphate buffer when the 2-ultra method was used, and with the same amount of distilled water when the heat-ultra method was used. Each homogenate was centrifuged at about 1,500 × gravity for 5 minutes to remove very coarse material. Various dilutions of the infective homogenate were then made by adding different volumes of non-infective homogenate. Each dilution was divided into 3 equal samples, and the virus recovered from a given volume of each sample.

In Figure 2, data from all of these experiments are plotted, as well as experiments described below, on the recovery of purified virus. Most of the points in Figure 2 represent the average of 3 determinations. An ideal curve would be a horizontal line. An inspection of the curves for "dilutions of infective homogenate" shows that the heat-ultra method produces more nearly horizontal curves and a more constant percentage recovery at different virus concentrations than does the 2-ultra method. At virus concentrations below 0.9 mg. per gram of tissue, the reliability of each method is distinctly less than at higher concentrations.[5]

[5] This lower limit of reliability is probably due to a failure of the dilute virus suspension to sediment uniformly with high speed centrifugation. A longer high speed centrifugation or a centrifugation at 100,000–125,000 × gravity might give more reliable results with lower concentrations of virus . Essentially all routine assay work has involved concentrations of virus greater than 0.9 mg. per gm. of tissue; the heat-ultra method has accordingly proved to be applicable in most of the virus assays.

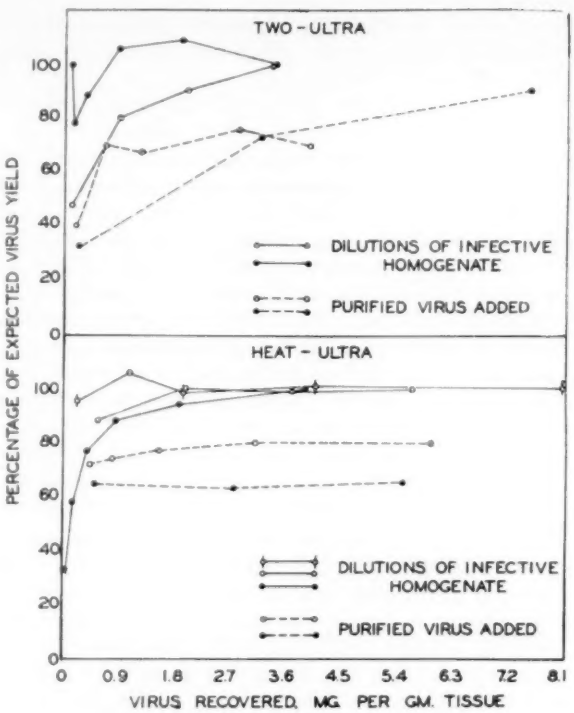

FIG. 2. Percentage of virus recovered by the 2-ultra method (upper graph) and by the heat-ultra method (lower graph) showing the greater uniformity of virus yield obtained by the heat-ultra method.

It must be pointed out that in the case of the "dilutions of infective homogenate" the recovery at the highest virus concentration is, for the purpose of calculation, taken as 100 per cent and the calculation of percentage recovery of the lower concentrations is based upon this. In the experiments with purified virus, described next, the percentage recovery represents the actual percentage of the added virus that was recovered.

A second similar series of experiments was conducted to compare the 2-ultra and heat-ultra methods with regard to the recovery of different concentrations of purified virus which had previously been mixed with homogenates from healthy tobacco leaves. Purified TMV was prepared by alternate cycles of low and high speed centrifugation at pH 7.0 in 0.1 molar phosphate buffer. Healthy frozen tobacco leaves were macerated in a mortar, the juice pressed out, and 1 volume of the plant juice mixed with 9 volumes of each dilution of purified virus. The purified virus dilutions in 0.1 molar pH 7.0 buffer were used in the 2-ultra method and dilutions in 0.01 molar buffer were used in the heat-ultra method (pH of mixture was about 5.6). In each experiment 3 replicates at each virus concen-

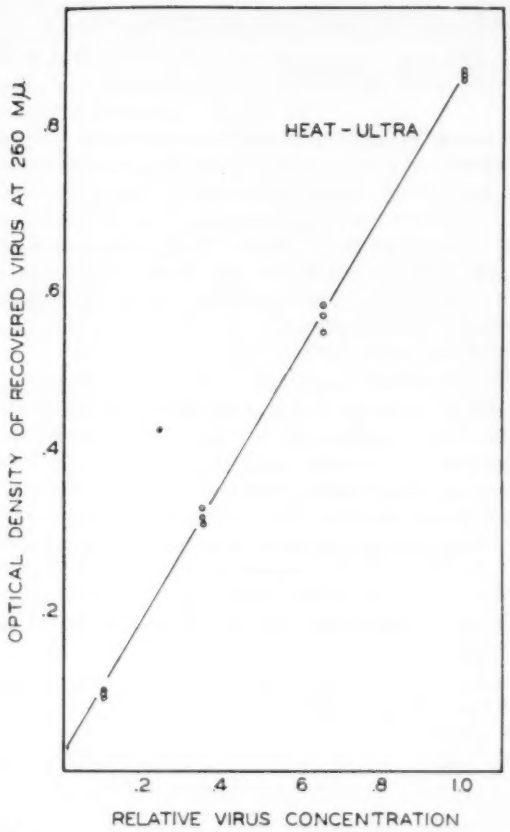

FIG. 3. The small variation among replicate assays. Each point represents a single assay on a dilution of a virus homogenate. The figure also demonstrates the linear relationship between dilutions and the optical density obtained.

tration were assayed by both methods and the percentage virus recovered was determined. Average results with the 3 replicates are plotted as points on the graph in Figure 2. The curves are designated on the graph as "purified virus added." The curves show that the heat-ultra method again gives a more constant percentage recovery of virus at different concentrations than does the 2-ultra method. The 2-ultra method shows a distinct tendency to produce a low percentage recovery as the virus concentration decreases. This is particularly marked at concentrations below 0.90 mg. of virus per gm. of tissue.

A study of the variation in replicates was also made. A graphic representation of typical variation in replicates of various dilutions of a mosaic homogenate is given in Figure 3. In this figure each point represents the result of a single assay. There are 3 replicate assays of each dilution of the infective homogenate. In preparing this graph the mean optical density of the virus recovered from the dilution containing the highest virus concentration was recorded as a point at the upper right end of the graph. A straight line was drawn from this point to the purified healthy homogenate reading near the lower left corner. If the same percentage recovery occurs at all virus concentrations, the optical density for all the dilutions would be expected to fall on this line. All the replicates fall close to the straight line, giving graphic evidence of the low variation among replicates as well as further evidence of the constant percentage recovery of the virus present in different dilutions of homogenates.

Calculations from the results of all the experiments showed that the average deviation of replicates from the corresponding mean optical density of each set averaged 2.3 per cent when the heat-ultra method was used, and 6.2 per cent when the 2-ultra method was used. Only those assays in which the virus concentration was above 0.90 mg. per gm. of tissue were used in making these calculations. The percentage deviation from the mean was greater when lower virus concentrations were assayed. Results reported show that when the virus concentration is above 0.90 mg. per gm. of tissue both methods have sufficient accuracy for most assay work; the heat-ultra method gives a more constant percentage recovery at different virus concentrations and less variation among replicates.

Under exceptional, not well understood conditions, the tobacco plant sap contained an unusual amount of brown material which was very difficult to separate from the virus. Mosaic sap containing excessive amounts of this material could not be satisfactorily assayed by either of the spectrophotometric methods because of the high absorption in the ultra-violet range by the brown material. Older plants and those grown under high light intensity appear most likely to contain excessive amounts of this brown material. Most of the plants used in these investigations yielded a final virus pellet with little or no color. The ultra violet absorption curve of the virus purified by the heat-ultra method gave further evidence of the high purity of the final product. This curve was usually indistinguishable from that of a virus purified by a number of cycles of centrifugation.

SUMMARY

Two methods of tobacco mosaic virus purification prior to spectrophotometric assay were compared. Alternate cycles of centrifugation were found to be less satisfactory than a method involving heating the homogenized tissues to coagulate impurities, followed by a cycle of low-high-low speed centrifugations. Of the 2 methods, this heat-ultra method recovers a more constant percentage of the virus from homogenates over a wider range of virus concentration; it also gives less variation among replicates from a particular virus homogenate. In experiments where the heat-ultra method was used the average deviation of replicates from the corresponding mean optical density was only 2.3 per cent. This indicates that the method is one of the most accurate of the assay technics for tobacco mosaic virus.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA

DESTRUCTION OF VARIOUS TISSUES IN CULTURE BY CERTAIN BACTERIA [1]

Zafrira Volcani,[2] A. J. Riker, and A. C. Hildebrandt

Tissue cultures are useful tools for studying not only basic metabolic activities of such tissues, but also reactions between these tissues and various microorganisms. Few attempts have been made to study the effect of bacterial plant pathogens on tissue cultures. Crown gall bacteria or their metabolites were examined by Hildebrandt, Riker, and Duggar (2), and by de Ropp (1). Similar host parasite relationships were studied for downy mildew of grape by Morel (7), tobacco mosaic virus by Kutsky and Rawlins (5), Kutsky (6), Hildebrandt, Riker and Watertor (3), and for cedar-apple rust by Hotson and Cutter (4). A pertinent review has appeared (8). Of interest, therefore, was a study of soft rotting bacteria on tissue cultures of several species.

MATERIALS AND METHODS.—The following microorganisms and strains were used:

Erwinia carotovora (Jones) Holland group: *E. carotovora* (W. J. Dowson, Cambridge, from celery); originally identified as *E. phytophthora* (W. J. Dowson, Cambridge, from potato).

Erwinia aroideae (Townsend) Holland (W. J. Dowson, Cambridge, from Uganda tobacco); *E. aroideae* (Z. Volcani, pepper fruit, Israel).

Erwinia atroseptica (van Hall) Jennison group:—*E. atroseptica* (W. H. Burkholder, N. Y., strain E. 1 from potato); *E. atroseptica* (Miss Noble, Scotland, from potato).

Bacillae group:—*Bacillus polymyxa* (Prazmowski) Migula (Z. Volcani, from *Capsicum*, Israel); *Bacillus subtilis* Cohn emend Prazmowski (Z. volcani, from *Capsicum*, Israel). These latter organisms have been found to be the cause of a fruit spot disease on *Capsicum annuum* in Palestine as well as to produce soft rot in various vegetables in the laboratory.

These various organisms were kept in culture for several years, except *E. atroseptica* (Scotland) which was isolated from potato plant in summer 1951. The pathogenicity of the organisms of the *E. carotovora*, *E. atroseptica*, as well as the *Bacillae* groups to vegetable slices in the presence of water was tested and proved to be very effective. The *E. atroseptica* group also caused black leg when inoculated on potato plants.

Carrot, marigold, periwinkle, and potato tissue cultures grown in 6 oz. bottles, 4 pieces in each bottle, were tested with the various microorganisms. Tissue cultures of the 3 former specimens were subcultures of stock maintained *in vitro* from 7 to 15 years and were free from contaminating bacteria. The potato tissue cultures were recent isolations from tubers, and inoculated after 3 successive transfers. One sample variety "Red Bliss" was supplied by S. M. Caplin, University of Rochester, N. Y.; the second was isolated by the senior author.

The methods of inoculation were as follows: a) 4- to 5-week-old cultures were moistened with a sterile-distilled-water suspension of 48-hour-old slant agar cultures of the various organisms respectively; b) a drop of the suspension of the organisms was deposited with a 3 mm. diameter loop on the top surface of the tissue without touching the nutrient medium in the bottle. The drop of the suspension was observed on the surface of the tissue for a few hours before it disappeared. This method was applied with carrot tissue only.

The tissue cultures in the controls were moistened with sterile-distilled-water instead of the suspension of the organisms.

The influences of bacterial metabolites from fermented media were studied with carrot tissue. The microorganisms were grown in liquid tissue culture medium for 4 to 5 days. The bacteria were then removed from the fermented medium by filtration, first through a Seitz filter and finally through a frittered glass filter. The pH of the medium was readjusted to its original value (about 6.0), and the medium distributed into bottles at the rate of 20 ml. of filtered medium to 10 ml. of fresh medium with agar sufficient to make 0.6 per cent. Four-week-old tissue pieces of carrot were transferred into the test bottles and controls, 4 tissue pieces in each bottle.

While carrot and potato tissues were inoculated with all the organisms listed above, marigold and periwinkle were inoculated only with 1 strain of *E. carotovora*, originally identified as *E. aroideae* (Cambridge), 2 strains of *E. atroseptica*, and the 2 *Bacillae*.

Both strains of the filtrate of the following organisms were tested with the carrot tissue: *E. aroideae*, *E. phytophthora* of the *E. carotovora* group; the 2 strains of the *E. atroseptica* group, and the 2 *Bacillae*.

Tissues inoculated with the organisms of the *Erwinia* group and their controls were kept at 26°C. Tissues inoculated with the *Bacillae* and their controls were kept at 32°C. These temperatures were at or near the optimum for growth both for the bacteria and for the tissue cultures. The tissue pieces transferred into the bottles with filtered medium were kept at 26°C. For each organism 3 to 4 bottles were used. Each experiment was repeated at least 2 or 3 times.

EXPERIMENTAL RESULTS.—*Effects of microorganisms*

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The photograph was prepared by Mr. Eugene Herrling.

[2] Plant Pathologist, on temporary leave from the Agricultural Research Station, Rehovot, Israel.

inoculated on tissue culture.—The various organisms induced, in most cases, marked softening and discoloration of tissues. However, there were some differences in the degree and progress of infection with a number of these organisms.

a) *Carrot tissue.*—Carrot tissues inoculated by moistening showed a definite discoloration with all the organisms; this appeared the third day following inoculation. It became more intense 3 to 4 days later. The tissues inoculated with *E. phytophthora*, however, discolored to a lesser degree. The organisms grew profusely on the medium, as well as on the tissue. A dislocation of lobes of the tissue was noticed first; this was followed later by gradual softening and shrinkage of tissue. Complete softening was produced with *E. aroideae* (Cambridge), *E. atroseptica* group, and with *Bs. polymyxa*. *Bs. polymyxa* alone produced complete disintegration of the tissue. *E. carotovora*, though inducing softening of potato and carrot slices in the presence of water, did not seem to cause any softening of the tissue in culture. (See Table 1 and Figs. A and B.)

Positive results also were obtained with carrot tissue inoculated by depositing a small drop of a suspension of the organisms on top of the tissue. Bacterial growth progressed gradually on the tissue. Three to 4 days following inoculation, some bacterial growth was also detected on the agar. Slight discoloration of tissue appeared on the 4th day after inoculation, which intensified 6 days later. Here again, entire softening of tissue was produced with *E. aroideae* (Cambridge), both strains of *E. atroseptica*, and *B. polymyxa*. *E. phytophthora* and *E. aroideae* (Israel) induced softening of upper layers only.

Microscopical examination of scraped soft tissue revealed numerous bacteria in, as well as between, the cells of the parenchymatous tissue. The vascular cells were free of bacteria in most cases.

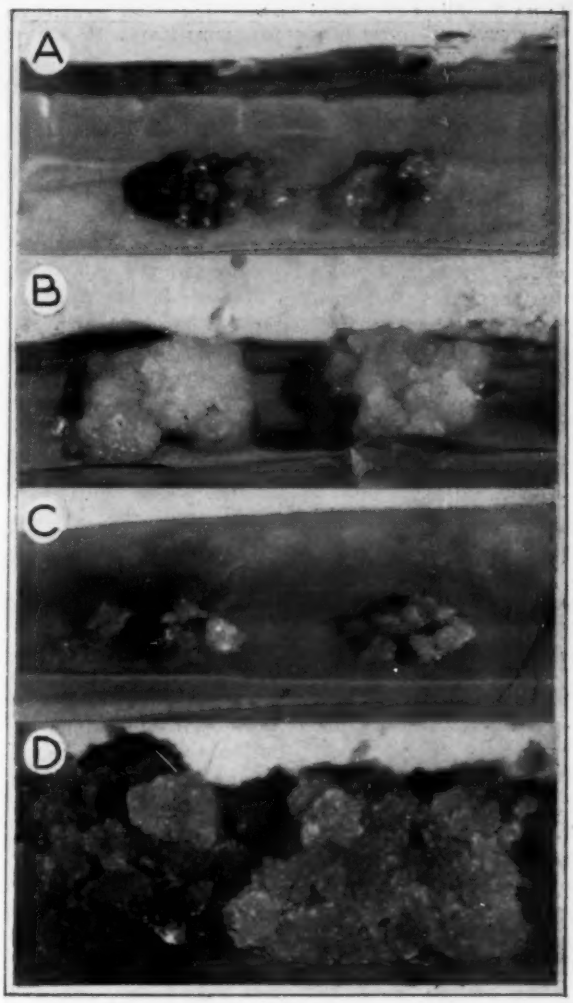

FIG. 1. Inoculated and control tissue cultures grown 4 weeks on 40 ml. of medium in 6 oz. bottles. A) Carrot tissue 14 days after inoculation with *E. aroideae* (Cambridge) at 26°C. B) Control specimen of carrot tissue at 32°C. C) Marigold tissue 14 days after inoculation with *B. subtilis* at 32°C. D) Marigold control tissue at 32°C.

TABLE 1.—*Inoculation experiments on carrot tissue with bacteria*

Cultures	Softening[a] Days 3	7	14
E. carotovora group			
E. carotovora[b]	0	0	0
E. phytophthora[b]	0	+	+
E. aroideae group			
E. aroideae[b]	+	++	+++
E. aroideae[c]	+	+	+
E. atroseptica group			
E. atroseptica[d]	+	++	+++
E. atroseptica[e]	+	++	+++
Bacillae group			
B. polymyxa[c]	+	+++	++++
B. subtilis[c]	0	0	0

[a]0 = none; + = slight; ++ = fair; +++ = entire; ++++ = disintegration.
[b]From Cambridge.
[c]From Israel.
[d]From U.S.A.
[e]From Scotland.

The control tissues, even those kept at 32°C., showed no discoloration. They remained perfectly healthy.

b) *Marigold.*—A profuse growth of all tested organisms appeared on the tissue inoculated by moistening it with sterile distilled water suspension of the organisms as well as on the medium the second day following inoculation. A slight discoloration was noted on the third day after inoculation and did not intensify markedly later. A total collapse of the tissue ensued on the 3rd day after inoculation, followed 2 to 3 days later by entire distintegration of tissue inoculated with *Bs. polymyxa*. (Figs. C and D.)

c) *Potato tissue.*—The inoculated potato tissues showed a definite discoloration with all the organisms

TABLE 2.—*Effect of filtered bacterial fermented medium on growth of carrot tissue in comparison with control specimens.*

Cultures	Total pieces transferred	Growth after 3 weeks	
		Slight	Good
		No.	No.
E. carotovora group			
E. phytophthora[a]	12	8	0
E. aroideae group			
E. aroideae[a]	12	1	0
E. aroideae[b]	8	2	0
E. atroseptica group			
E. atroseptica[c]	12	3	0
E. atroseptica[d]	16	1	0
Bacillae group			
B. polymyxa[b]	16	3	0
B. subtilis[b]	16		0
Control	16	0	16

[a]From Cambridge.
[b]From Israel.
[c]From Scotland.
[d]From U.S.A.

on the second and third days following inoculation, with discoloration becoming more intensified 3 to 4 days later. The organisms grew well on the medium as well as on the tissue. Collapse of tissue was observed on the third and fourth days after inoculation, and was followed by disintegration in the case of *E. aroideae* (Cambridge) and *Bs. polymyxa*. Partial softening of the tissue was produced with the 2 strains of *E. atroseptica* and *E. phytophthora*. Softening of upper layers only was produced with *E. carotovora* and *Bs. subtilis*.

d) *Periwinkle.*—Signs of infection were also detected on the inoculated periwinkle tissue, but to a lesser degree than with both former tissue cultures.

Slight discoloration was noted on tissues inoculated with *E. aroideae* (Cambridge) and with the 2 isolates of *E. atroseptica*. Later also with those inoculated with *Bs. polymyxa*, softening of tissue was only partial. No softening occurred in tissues inoculated with *Bs. subtilis*.

Summing up the results of these experiments, it appears that the most effective microorganisms were *E. aroideae* (Cambridge) of the *E. carotovora* group, both strains of *E. atroseptica*, and *B. polymyxa* of the *Bacillae* group.

Effect of filtered bacterial fermented medium on carrot tissue growth.—A marked delay of growth, as well as difference in final size of tissue, was observed with the tissues grown on the filtered medium in comparison with the controls at 3 and 4 week incubations. In some cases no growth appeared.

In no case where growth occurred did carrot tissue, grown on filtered fermented medium, attain the size of that grown on basal media. The results of the first test 3 weeks following transfer are in Table 2. Similar results appeared with the second test.

SUMMARY

The effects of 6 bacterial soft rot organisms of the *Erwinia* genus (*E. carotovora* group, *E. aroideae*, and *E. atroseptica* group) as well as 2 *Bacillae* (*B. subtilis* and *B. polymyxa*) were tested on tissue cultures. Carrot, periwinkle, potato, and marigold were inoculated by moistening the tissue pieces with sterile distilled water suspension of these organisms. Six of these 8 microorganisms produced a distinct discoloration and softening of the carrot and potato tissue, and entire collapse of the marigold tissue. Periwinkle tissue was affected less. The degree of infection and its progress varied, however, with the different organisms. *B. polymyxa* caused the most devastating effect: entire disintegration of carrot, potato, and marigold tissue. *E. carotovora* and *B. subtilis* appeared the least effective.

The metabolites in the fermented media from these microorganisms induced delay in growth and decrease in size of carrot tissue.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

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A HISTOLOGICAL STUDY OF THE INFLUENCE OF SPROUT INHIBITORS ON FUSARIUM INFECTION OF POTATO TUBERS [1]

H. S. Cunningham

Examination of potato tubers in storage indicated that, in many instances, Fusarium dry rot was more prevalent in tubers treated with a sprout inhibitor than in untreated tubers stored under the same conditions. Subsequent studies (4) have shown that the percentage of rotted tubers in ordinary storage is significantly higher when certain types of sprout inhibitors are used than in untreated tubers. This observation naturally raised the question as to whether the higher amount of rot was due to the active sprout inhibiting agent, or to 1 or more of the inert ingredients used either as a carrier or coloring agent in the product as marketed. It seemed quite possible that some component of the sprout inhibiting mixture might prevent, or delay, the healing of wounds and thereby facilitate entrance of Fusarium.

Although Bisby (2) states that *Fusarium discolor var. sulphureum* (Schl.) App. (*F. sambucinum* Fuck. f.6 Wr.) may sometimes enter through uninjured surfaces, probably through lenticels, the author (3) has never succeeded in infecting potato tubers with this organism through the uninjured natural skin nor through a properly healed cut surface.

Healing of wounds in potato tissue is essentially a growth process and, as shown by Priestly and Woffenden (5) follows a definite sequence. Under normal conditions suberization of the walls of cells near the outer surface proceeds rapidly until there is a continuous suberized layer 2 or 3 cells in depth. The cells lying immediately beneath the suberized area soon lose their starch content and by division give rise to a layer of actively dividing cells lying roughly parallel to the outer surface. Cell division continues until there is a continuous layer of tabular cells closely joined together without intervening air spaces. In time the walls of the outer cells in this layer also become suberized.

Any condition which delays suberization and subsequent wound cork formation, without seriously inhibiting the growth of the organism, will result in penetration of the tissues to a depth where the tuber cannot react to form a protective barrier.

This study was undertaken to determine the effect of commercial sprout inhibitors upon the normal healing processes of potato tubers and to identify the ingredients of products responsible for increased prevalence of Fusarium dry rot in treated tubers.

METHODS.—The variety Green Mountain was used in this work. Seven-day-old cultures of *Fusarium sambucinum f.6* on potato-dextrose agar served as a source of inoculum. This species was used mainly because it is commonly found causing dry rot in storage, also because it readily produces spores on artificial media. Inoculum, consisting of spores suspended in water, was applied to the cut surface of the tubers with an atomizer until the surface was thoroughly wet. The cut surface was allowed to dry for a short time before sprout inhibitors were applied.

Liquid sprout inhibitors also were applied with an atomizer. Dusts were either shaken from a cheese cloth bag onto the cut surface or applied by means of a settling tower.

Where high humidity was required the cut tubers were kept in moist chambers. Under these conditions the humidity was at, or near, saturation at all times. There was no forced circulation of air through these moist chambers but they were not completely air tight. Each day covers were removed for a short time when samples were taken.

In experiments where lower humidities are reported the cut tubers were placed in open containers where the humidity equalled that of the storage chamber.

Each day, for 10 days, samples were taken from the periphery of each tuber. Small sections were cut from these samples, fixed in chromo-acetic solution (½ per cent each), washed, and run up into paraffin in the usual manner. All sections were cut at 25μ and attached to the slide with Land's fixative. Ammoniacal gentian violet was used as a stain for suberin. Cotton blue and a counter stain of Orange-G were used to distinguish mycelial growth in the tissues.

Series A.—All tests in this series were kept at high humidity and at a temperature of 65°F–70°F. Three commercial sprout inhibitors were used, 2 in dust form (AD[2] and BD[3]) and 1 in liquid form (BL[2]). The active agent in these 3 sprout inhibitors was the methyl ester of naphthaleneacetic acid. In addition, the pure methyl ester was used as a smear over the cut surface of tubers and also in volatile form. In the latter case the methyl ester was placed in a watch glass within the moist chamber. In this series the sprout inhibitors were used in excess of the dosage recommended by the manufacturer.

Effect of treatments on noninoculated tubers.—Where no treatment was applied to the cut surface of the tuber, suberization of the walls of the outermost cells was noted on the day after the experiment was set up. The suberin that appeared the first day was irregular in distribution but by the second day a com-

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The author is indebted to the American Cyanamid Company, the Grange-League-Federation, and the Sterling-Winthrop Research Institute for supplying materials used in this investigation.

[2] Methyl ester of naphthaleneacetic acid the active agent (Barsprout).

[3] Methyl ester of naphthaleneacetic acid the active agent (Dorm-Aid).

plete layer of suberized cells was present. Cell division, the beginning of wound cork formation, was apparent in some cells directly below the suberized area. On the fourth day wound cork was present in a continuous layer. The wound cork continued to form until the layer was several cells in depth. Toward the end of the experiment the outermost cells in the wound cork also became suberized. This may be considered normal healing under the conditions of the experiment.

Where treatment AD was used, irregular suberization was present 4 days later than normal and no evidence of a complete suberized layer was found during the period of the experiment. There was no evidence of wound cork formation. The response to treatment BD was somewhat different. Irregular suberization was noted on the third day but not until the eighth day was a complete suberized layer formed and at this time wound cork was forming irregularly. With treatment BL, suberization was delayed until the fifth day and was irregular throughout. No wound cork was formed.

When the pure methyl ester was smeared on the cut surface the healing process was wholly subdued. The healing process was delayed 72 hours by the methyl ester in volatile form but proceeded normally thereafter. The inert ingredients used in making up dusts AD and BD had little or no effect on the healing process under the conditions of this experiment.

Effect of treatments on infected tubers.—In general, the presence of *Fusarium* in potato tissue caused a delay in the healing process and the suberized layer was situated somewhat deeper than normal.

Where tubers were untreated, or treated with inert materials, the healing process was delayed from 2 to 3 days. Once healing was initiated it proceeded normally and a barrier formed which effectively sealed off the organism within the outer cells.

Suberin formation was delayed by treatment with dusts AD, BD, and the liquid BL and occurred irregularly during the period of the experiment. No wound cork formed. The *Fusarium* continued to penetrate the tissues and, in the case of dust AD, the tubers were so far decayed that samples could not be taken after the eighth day.

Where the pure methyl ester was smeared on the cut surface, the healing process was entirely subdued. The *Fusarium* grew luxuriantly on the surface and rapidly penetrated the tissues. Decay was nearly complete in 10 days. The methyl ester used in volatile form resulted in irregular suberin formation with evidence of wound cork on the ninth day. Growth of the fungus was in no way inhibited.

Series B.—All tests in this series were run in triplicate. One lot was kept in moist chambers where the humidity was at, or near, the saturation point at all times. The other 2 were kept in open containers where the humidity was that of the storage chambers. This was 85 per cent relative humidity in 1 chamber and 40 per cent in the other. The temperature in these storages was the same and varied from 52°F.–60°F. Two commercial sprout inhibitors in dust form (AD and CD[4]), and 1 experimental lot in liquid form (ML[5]) were used. The dust was applied by means of a settling tower. None of these materials was used in excess of the amount recommended by the manufacturer.

Effect of treatments on noninoculated tubers.—The low temperature used in this experiment delayed healing as evidenced by the fact that suberin and wound cork in untreated tubers at high humidity appeared 48 hours later than in Series A. Figure 1,A illustrates the extent of wound cork formation in untreated tubers on the eighth day.

Treatment with dust AD did not affect suberization. Wound cork, however, was formed irregularly and did not form a continuous layer (Figure 1,C). Complete suberization was delayed still further by treatment with ML, and here also wound cork was formed irregularly. Dust CD did not delay the initial formation of suberin but suberin formation was irregular for a longer period of time. The CD dust did cause delay in the appearance of wound cork (Figure 1,E).

Humidity had a direct effect on the healing process. Low humidity resulted in drying of the outer cells. The lower the humidity the deeper this area extended into the tissues. At 40 per cent relative humidity, suberin eventually appeared in cell walls below this area in both treated and untreated tubers. Irregular wound cork later formed in untreated tubers and also in tubers treated with CD. Suberin was deposited irregularly where treatments AD and ML were used but no wound cork formed.

Effect of treatments on infected tubers.—At high humidity, all treatments, with the exception of CD, delayed to some extent the first appearance of suberin in the cell walls. A continuous layer of suberized cells was not evident until the eighth day (Figure 1; B,D,F). If wound cork formed it followed an irregular pattern except with untreated tubers and with treatment CD.

At 40 per cent relative humidity, treatment with ML suppressed all healing. No wound cork formed except with treatment CD and then not until the tenth day.

None of the treatments affected in any way the growh of *Fusarium*. Penetration of the organism persisted until a continuous layer of wound cork was formed. Although irregular formation of wound cork occurred somewhat later with treatment CD than in untreated tubers, a continuous layer was present on the same day. As a result the amount of rot was no greater with treatment CD than in untreated tubers.

Discussion.—Priestly and Woffenden (5) showed that a continuous layer of suberized cells is essential for the formation of a complete layer of wound cork. Apparently it does not follow, that the formation of a continuous layer of suberized cells will necessarily result in the formation of a complete layer of wound cork. If sprout inhibitors are applied to the cut surface of potatoes, wound cork may be formed irregu-

[4] CD Tetrachloronitrobenzene the active agent (Fusarex).
[5] ML Methyl ester of naphthaleneacetic acid the active agent (experimental lot).

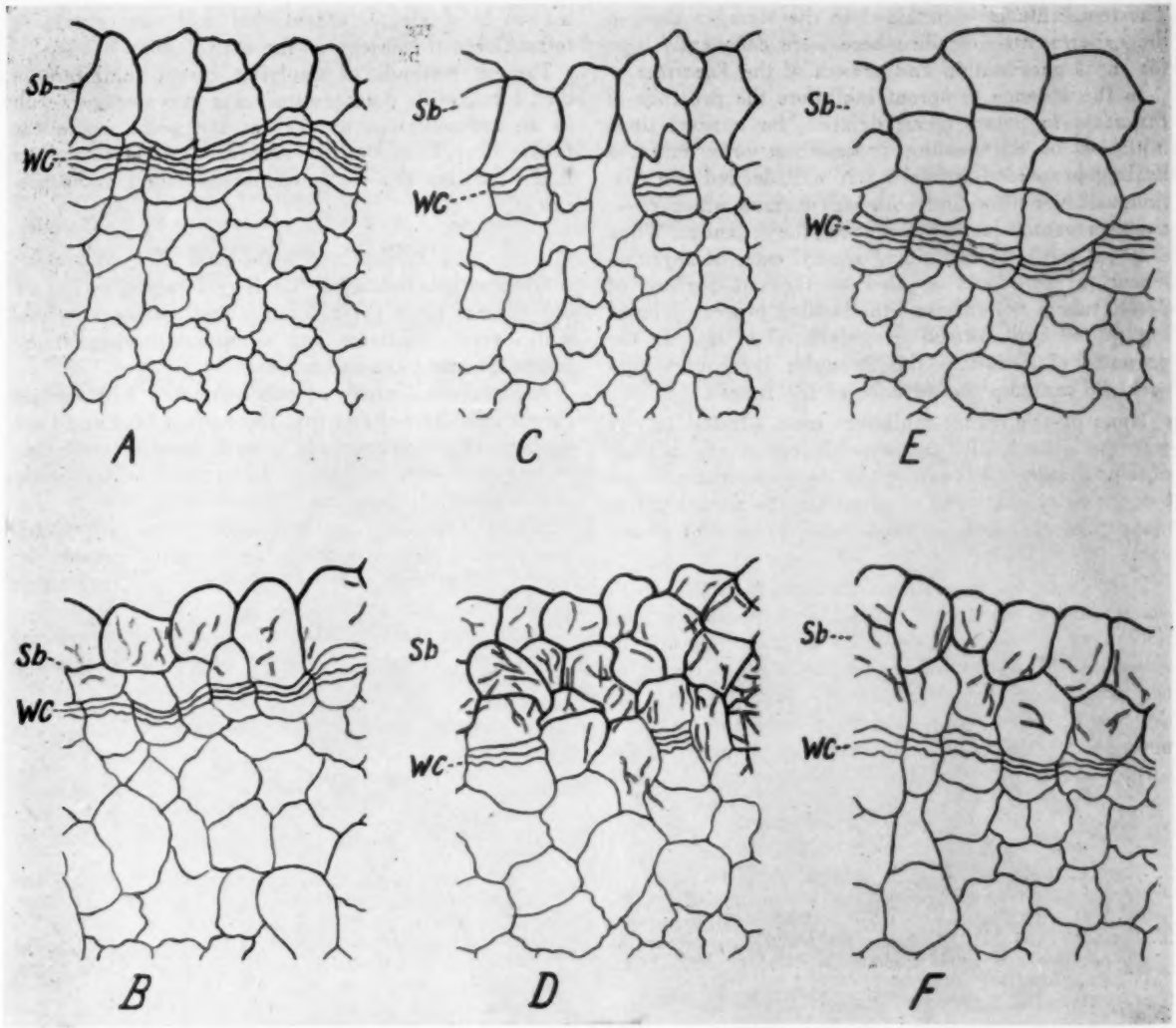

FIG. 1. Influence of certain sprout inhibitors on healing of cut surfaces of potato tubers in saturated atmosphere and the effect on penetration of *Fusarium* 8 days after treatment. A,B) Not treated; C,D) methyl ester of naphthaleneacetic acid; E,F) Tetrachloronitrobenzene. Sb., suberin; WC, wound cork.

larly or not at all, even when a continuous layer of suberized cells is present. Formation of wound cork is essentially a growth process and sprout inhibitors might be expected to affect this form of growth as well as prevent development of sprouts.

Weiss, Lauritzen, and Brierly (6) state that suberized cells are not in themselves a barrier against penetration of *Fusarium* and not until a well defined layer of wound cork is present is a complete barrier against further advance of the organism established. In all the infected tubers examined in connection with the work reported in this paper *Fusarium* was found to be present in the suberized cells and continued to penetrate the suberized layer until wound cork was formed. In no instance was Fusarium found below a well defined and continuous layer of wound cork.

Artschwager (1) has shown that rapidity of the healing process depends on temperature and humidity. If the humidity is low the injured surface of the tuber will dry out and crack, whereas at high humidity the suberization rate is dependent upon storage temperature. If the temperature is very low suberization will be greatly delayed whereas at high temperature and high humidity suberization becomes noticeable after the first day. In the work reported here the cut surface of the tubers dried out and cracked at 40 per cent relative humidity and suberization occurred deep in the tissues. The humidity was not low enough to prevent infection nor to slow up growth and penetration of *Fusarium*.

The effect of temperature on germination of spores of *Fusarium sambucinum f.6* has been noted by the author (3). At 9°C spore germination required 48 hours although at 18°C it occurred in only 9 hours. Growth on agar plates as measured by the diameter of the colonies was twice as rapid at 18°C as at 15°C.

The temperatures maintained in the storages used in the experiments described here were sufficiently high for rapid germination and growth of the *Fusarium*.

In the absence of sprout inhibitors the presence of *Fusarium* in potato tissue delayed, for a short time, initiation of the healing process but once initiated healing proceeded normally. A well defined and continuous layer of wound cork was formed which effectively prevented further advance of the organism. When a sprout inhibitor containing methyl ester of naphthaleneacetic acid was applied to the cut surface of potato tubers not only was the healing process delayed but wound cork formed irregularly, if at all. In the presence of *Fusarium* this irregular layer of wound cork did not stop the advance of the fungus.

None of the sprout inhibitors used affected in any way the growth of *Fusarium*. Infection was readily obtained under all conditions in these experiments and the organism continued to penetrate the tissues unless stopped by the formation of a complete layer of wound cork.

Data presented here show that sprout inhibitors will delay, and even inhibit, wound cork formation and not prevent infection by or subsequent growth of *Fusarium*. If sprout inhibitors are used in excess of the amount recommended by the manufacturer the detrimental effect on wound cork formation is more marked. A sprout inhibitor containing methyl ester of naphthaleneacetic acid will inhibit wound cork formation to a greater extent that will one containing tetrachloronitrobenzene as the active agent.

Present methods of applying sprout inhibitors in dust form, while potatoes are going into storage, result in an accumulation of dust at the point where the tubers drop from the bin loader. It is at this point that Fusarium dry rot is commonly found to be prevalent.

SUMMARY AND CONCLUSIONS

Observations indicated that dry rot caused by *Fusarium sp.* was more prevalent in stored potatoes treated with a sprout inhibitor than in untreated tubers stored under the same conditions.

A histological study of tubers treated with certain sprout inhibitors shows that the normal healing of cut surfaces of potato tubers is delayed without interfering in any way with growth of *Fusarium*. Wound cork, if formed at all, is formed irregularly.

A sprout inhibitor in which methyl ester of naphthaleneacetic acid is the active agent has a greater delaying action than one having tetrachloronitrobenzene as its active agent.

The active agent contained in sprout inhibitors was found to be responsible for any delay in the healing of wounds.

DEPARTMENT OF PLANT PATHOLOGY
CORNELL UNIVERSITY
ITHACA, NEW YORK

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CONCENTRIC CANKER AND WOOD ROT OF CITRUS ASSOCIATED WITH FOMES APPLANATUS IN FLORIDA [1]

James F. L. Childs [2]

A disease of citrus trees called wood rot (presumed to be due to 1 or more basidiomycetes) has been recognized [3] in Florida for many years, but the form here designated as concentric canker that is commonly associated with it and which the author regards as a phase of the same disease appears not to have been recognized previously. It is of rather frequent occurrence on old trees of grapefruit (*Citrus paradisi* Macf.), sweet orange (*C. sinensis* (Linn.) Osbeck) and Dancy tangerine (*C. reticulata* Blanco). In a 50-year-old orchard of Duncan grapefruit near Orlando, over 19 per cent of the trees were affected with the canker form of the disease. Twenty per cent more trees showed evidence of wood rot without the bark

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[2] Pathologist, Fruit and Nut Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

[3] Suit, R. F. 1949. Parasitic diseases of citrus in Florida, University of Fla. Agr. Exp. Sta. Bul. 463, 112 pp.

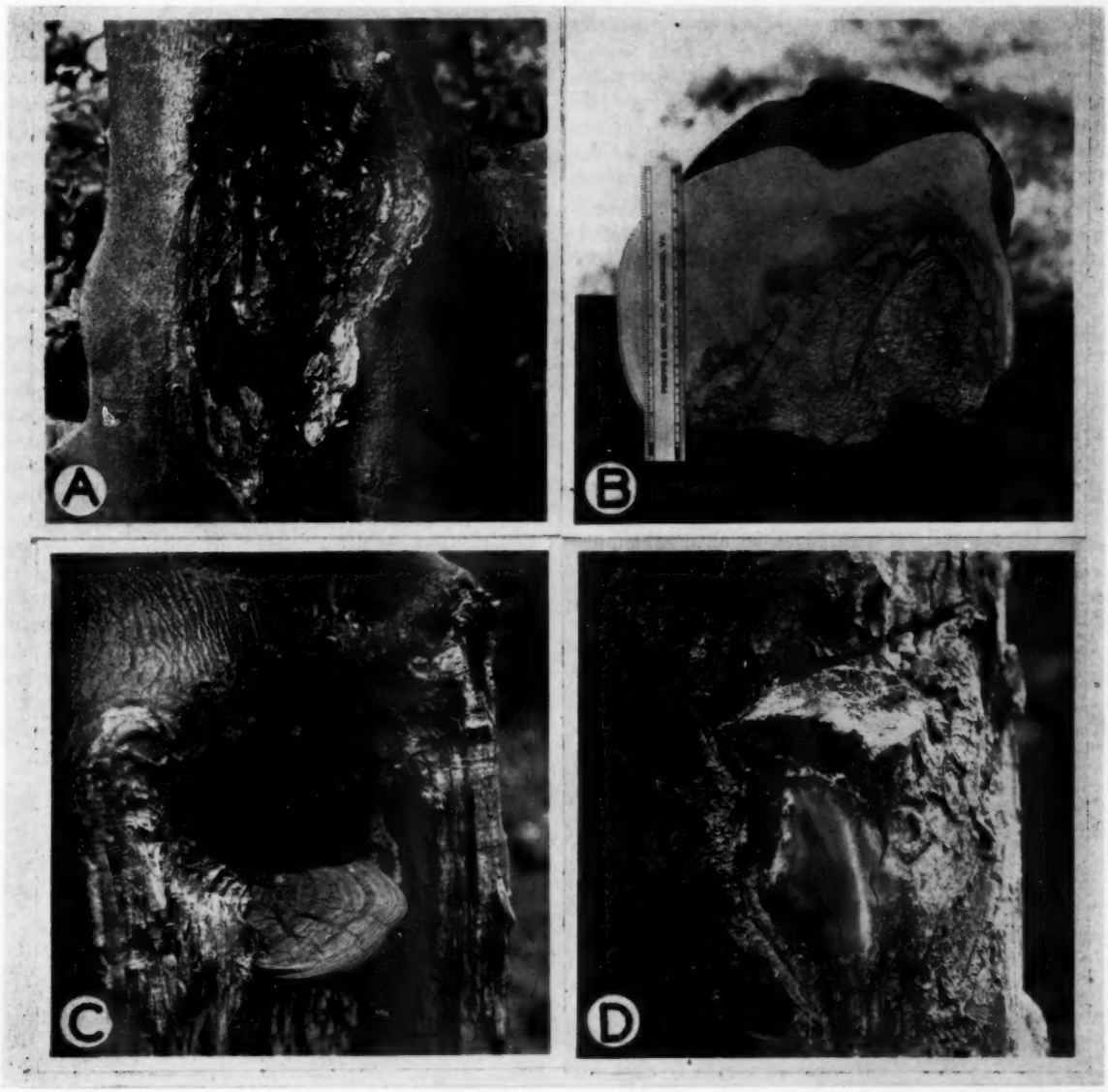

FIG. 1. A) Concentric canker on a 50 year old Duncan grape fruit tree. Knife marks the point of infection, a partially overgrown stub; B) Young and old wood is decayed, eventually becoming soft and "punky", and the bark is killed; C) Sporophore of *Fomes applanatus* on Duncan variety of grapefruit; D) Resupinate sporophore of *F. applanatus* on an old branch stub. Same tree as B.

canker symptom. Although the term heart rot of citrus is occasionally used, the term is rather indefinite since citrus wood has no distinct heart.

SYMPTOMS.—Wood rot where visible is easily recognized but in the absence of visible signs of wood decay the concentric type of bark canker is often an indicator of infection. The size and position of the cankers vary with the age and the point of infection but in general cankers are lenticular in outline with the long axis parallel to the axis of the trunk or branch (Fig. 1, A). The bark is broken by more or less concentric cracks or fissures that probably represent previous margins of the canker. A dead stub or a wound is usually found at the center of the cankered area and doubtless was the point of original infection (Fig. 1, D, and at knife point in Fig. 1, A). The dead bark within the limits of the cankered area is usually shaggy or scaly.

Those portions of wood long affected by decay are quite "punky" in consistency, but the more recently invaded wood is of about the same hardness as healthy wood and is tan to brown in color. The wood near the bark seems to be invaded no less readily than the older wood towards the center of the trunk or branch (Fig. 1, B). This, together with the fact that the bark is killed, indicates that the causal organism is a true pathogen on citrus.

Sporophores of a basidiomycetous fungus are usually but not always associated with the concentric canker and wood rot. These have been tentatively identified as sporophores of *Fomes applanatus* (Pers.) Wallr. (Fig. 1, C), and conform to Stevens' description[4] of sporophores of this species.[5] Their context is deep reddish-brown and the margin or lip is cream or white. The largest sporophore found measured 16 in. across the widest portion but most are smaller, 3–6 in. across. Occasionally a sporophore on an overhanging surface is resupinate in form (Fig. 1,D). Although *F. applanatus* is usually regarded as saprophytic on the heartwood of various hardwoods and conifers, Boyce[6] mentioned that it does attack living hardwood trees and according to Stevens[4] Heald reported it as a parasite on living cottonwood trees.

Portions of the fungus sporophore have been cultured on potato-dextrose agar and inoculations have been made into mature grapefruit trees. However, it may be a number of years before data are available that confirm or disprove the implications evident in the continual association of *F. applanatus* sporophores with this concentric type of bark canker and wood rot.

ECONOMIC IMPORTANCE.—This disease is not a limiting factor to the citrus industry but it is unquestionably of considerable importance in the general deterioration of citrus orchards under Florida conditions. Because of its wide distribution and the unobtrusive nature of its attack, wood rot, together with its less known phase of concentric canker, causes much more damage to citrus trees than is generally recognized. As a result the disease merits cognizance and consideration by production managers and grove caretakers particularly since prevention is such a simple matter.

CONTROL.—The control of this disease is largely a matter of prevention. Early infections can be excavated, and the wound disinfected and coated with a suitable wound dressing, but excavation of the more extensive infections may weaken a tree so much that it is easily damaged by high winds or hurricanes. Prevention is accomplished by following the principles of good pruning practice. Dead or broken branches should be cut off flush, leaving no stubs or tag-ends that would be callused over with difficulty (See Fig. 1. D). All wounds should be disinfected and then painted with a water-repellent wound dressing. Avenarius or Red Arrow carbolineum has been found most satisfactory as a wound disinfectant because it penetrates into the wood much better than aqueous disinfectants. One of the asphalt emulsion preparations makes an excellent wound coating that does not crack or become pervious to water after exposure to the weather.

U. S. SUBTROPICAL FRUIT FIELD STATION
ORLANDO, FLORIDA

[4] Stevens, F. L. 1913. The fungi which cause plant disease, The Macmillan Co., New York, 754 pp.

[5] Identification of *Fomes applanatus* was confirmed by J. A. Stevenson of Mycology and Plant Disease Survey Div. of Bureau of Plant Industry, Soils, and Agricultural Engineering of U.S.D.A. who wrote Aug. 11, 1952, "We have a specimen of this species on *Citrus limonia* from Calif. but no published records. Teodoro in Enumeration of Philippine Fungi, p. 309, 1937, lists a Reinking specimen on citrus and presumably Reinking in one of his lists of Philippine Fungi does likewise. Further than this I find no published records."

[6] Boyce, J. S. 1938. Forest pathology, McGraw-Hill Book Company, New York.

AN ACTINOMYCETE ASSOCIATED WITH GUMMOSIS DISEASE OF GRAPEFRUIT TREES [1]

James F. L. Childs [2]

Beginning in 1907 Fawcett (3, 4, 5, 6) described gummosis disease of citrus trees in Florida. Apparently the same disease was recognized as "lemon gum disease or gummosis" in California by Smith and Butler (12) in 1908, when they clearly distinguished gummosis from foot-rot, a disease caused by *Phytophthora* spp., with which its has since been confused. In 1931 Rhoads and DeBusk (10) recognized gummosis, in descending order of severity, on lemon (*Citrus limon* (Linn.) Berm.), grapefruit (*C. paradisi* Macf.), sweet orange (*C. sinensis* (Linn.) Osbeck), and tangerine (*C. reticulata* Blanco), but considered sour orange (*C. aurantium* Linn.) very resistant if not immune. Although Fawcett (6) isolated *Diplodia natalensis* from gummosis lesions a number of times, he reported that typical gummosis symptoms did not result from inoculation with it. Five years of work with *D. natalensis* and *Phomopsis citri* convinced Stevens (11) that neither was a primary cause of gummosis. More recently Godfrey (8) reported gummosis as a new disease (Rio Grande gummosis) on grapefruit trees in Texas. That the symptoms of the Texas disease do not differ from those of Florida gummosis was pointed out in another paper (2). Godfrey (8) noted that an actinomycete-like fungus was associated with gummosis. He also noted that *Diplodia* was often associated with the disease but considered it a secondary invader. Olson (9) reported, however, that when inoculated into Texas grapefruit trees, certain isolates of *Diplodia* produced pink (salmon) staining of the wood, a symptom associated with gummosis. Thus, there are those who consider a species of *Diplodia* in all probability the primary cause and others who do not. The purpose of this paper is to call attention to certain undescribed features of the pathological anatomy of gummosis-affected graprefruit trees because of the bearing they are considered to have on the nature of the causal agent.

GUM POCKETS.—The most striking symptom of gummosis, in addition to bark scaling, exudation of large quantities of gum, and salmon-colored staining of the wood, is the presence of gum pockets of a peculiar type in the wood, usually ¼ in. or more beneath the cambium. So far as is known to the author, gum pockets of this type are not associated with any other disease of citrus. C. Waibel of Texas [3] has asserted that gum pockets are also associated with psorosis disease, but Webber and Fawcett (13), Fawcett and Bitancourt (7), and Bitancourt et al (1) did not mention them. In the author's opinion the lesions in psorosis-affected citrus wood are not comparable to the gum pockets of gummosis as the former show none of the features hereinafter described.

The manner in which gum pockets arise is not known with certainty. In some cases they appear to start when, as a result of local infection through the bark, gum instead of a layer of wood is formed in the cambial region. Subsequent layers of wood laid down over the affected area thus would form a thin pocket of gum. At other times a slight overgrowth or gall is formed at the point of infection in the cambial region and gum is not immediately formed. In Fig. 1, A, the bark has been removed from 2 adjacent, very young natural infections. On the left a shallow layer of gum has been formed; on the right a very slight overgrowth is discernible. Small gum pockets are commonly found underneath such overgrowths. The cambial face of the gall-like process (xylem) is often greenish, presumably due to formation of chlorophyll in the pathological tissues. Frequently gum pockets appear to have been formed within the xylem as a result of infection that had penetrated axially from some distant point of entry such as a pruning wound.

GALL TISSUE.—Within the gum pockets, small tubercle-like outgrowths or galls, arising from the adaxial and abaxial walls enlarge and fill the cavity. Three excised gum pockets cut in a radial plane to expose gall-filled pockets are shown in Fig. 1, B. The leftmost piece has had part of the cambial face cut away. Face views of the tubercle-like out-growths on abaxial walls of excised gum pockets are shown in Fig. 1, C. These gall-like growths (Fig. 1, B, and C) are stimulated presumably by some metabolic product of the causal organism. Gall-filled gum pockets in the wood are usually indicated by risings (due to uplifted layers of wood and bark) on trunk or limb. At the same time that gall tissue is proliferated, portions of the wood and gall tissue appear to be dissolved, presumably by enzymatic action (10), giving rise to channels of gum throughout the mass (Fig. 1, D). The wood adjacent to gum pockets (Fig. 1, B) may be of normal pale yellow or of an unhealthy buff color streaked with salmon-colored bands. Occasionally the characteristic black mycelium of *Diplodia natalensis* has been found associated with gum pockets, but the reverse situation occurs often enough to cast doubt on the causal significance of that fungus.

ACTINOMYCETE PRESENT.—In every gum pocket examined an actinomycete has been found abundant in the gum channels between masses of gall tissue but its demonstration has presented some difficulties. For this purpose thin sections were planed from the radial (or tangential) face of an excised gum pocket or portion thereof, using a No. 60½ Stanley block plane

[1] Accepted for publication November 20, 1952.

[2] Pathologist, Fruit and Nut Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

[3] In correspondence with author.

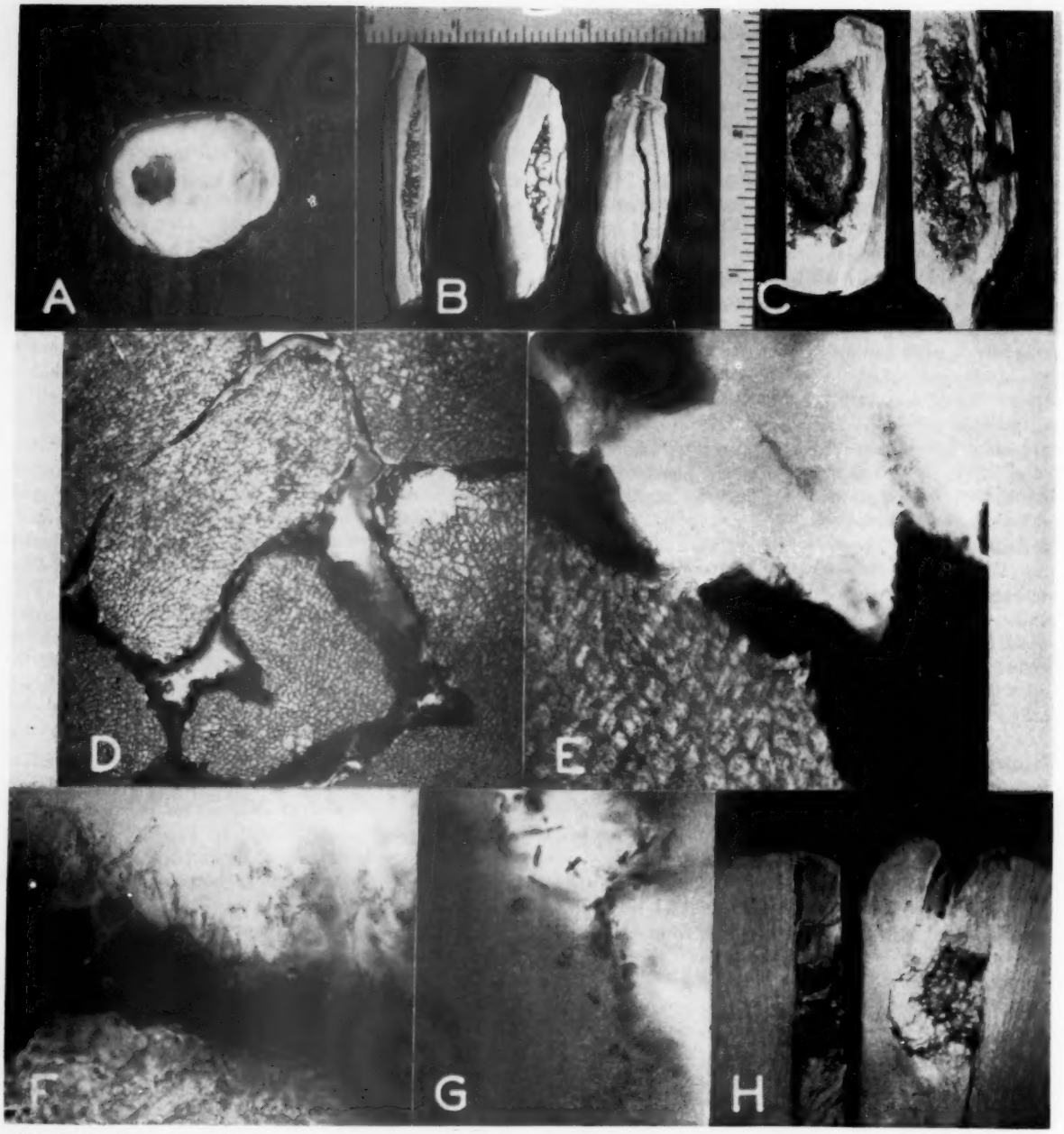

FIG. 1. A) Bark removed from 2 adjacent natural infections to show early stages of gum pocket formation, ½ natural size; B) Excised gum pockets in radial plane view; C) Internal surfaces (abaxial) of 2 gum pockets showing nodular gall tissue; D) Radial section of gum pocket gall tissue showing channels of gum and proliferating cells (approx. ×50); E) Actinomycete hyphae extending from gum into an open area (approx. ×260); F) Actinomycete hyphae (approx. ×540); G Actinomycete fragments (approx. ×1800); H) Gum pocket 16 months after inoculation, natural size.

with blade honed to razor sharpness. For the cutting of these sections a plane of this type was superior to most microtomes. Also fresh material was more satisfactory than dried because thinner sections could be cut. Without preliminary treatment the sections were flattened on a microscope slide and stained with cotton blue in lactophenol and a coverslip applied. A few air bubbles in the section were not a serious matter, but heating to remove air bubbles might cause the delicate mycelial strands to be dislodged and lost. Cotton blue stained the gum slightly but after 24 to 48 hours' contact the actinomycete picked up sufficient stain that it could be distinguished readily (Fig. 1, E). Cartweight's stain made a more striking preparation for low power examination but it stained the gum dark red. This made the blue-stained mycelium and mycelial fragments difficult to distinguish except in places where the gum was extremely thin.

The mycelial strands were about 0.5μ in diameter and of any length. Fragments ranged from about 1.5μ to about 3.0μ in length. Masses of the actinomycete embedded in the gum or extending into a clear area (Fig. 1, E)) could often be distinguished at a magnification of about 260×, but usually about 560× was required. Although the mycelial strands appeared to consist of a series of short, rod-like bacteria, in many instances pressure applied to the coverslip did not separate the rods; rather they hung together like a string of sausages. Rod-like mycelial fragments could also be demonstrated in the liquid gum of the cavity (Fig. 1, G).

Isolation of the actinomycete.—This organism was first isolated from gum pocket tissue taken from grapefruit in 1950, and a number of isolations have since been made from other gum pockets. It has been recovered twice from inoculations on grapefruit trees. Both egg-albumin agar and glucose-asparagin agar have been used for cultures but were not entirely satisfactory. The microorganism grows so slowly on these media that month-old isolation attempts should be examined at a magnification of 50× before deciding that they are sterile.

Inoculations.—Inoculation of this organism under the bark of McCarty grapefruit caused only very slight gumming in 1 month but when the bark was removed the wood was found to have developed at the point of inoculation a slight green overgrowth similar to natural infections through the bark. Duncan grapefruit trees examined 16 months after inoculations showed typical gummosis bark scaling, and removal of the bark revealed gall-like processes arising from the cambial face of the wood. About ¼ in. beneath one of the gall-like processes a typical gum pocket had been formed (Fig. 1, H). Slides cut from this gall tissue and smears of the gum revealed the actinomycete-like fungus. A similar appearing actinomycete was recovered in culture. *D. natalensis* was not present as indicated by examination and by failure to isolate it from the gum pocket tissues.

SUMMARY AND CONCLUSIONS

Masses of gall tissue with channels of liquid gum, the whole more or less deeply buried in the xylem, constitute the gum pockets that are a characteristic feature of grapefruit trees affected with gummosis. An actinomycete has been found regularly in the gum pockets. It has been isolated, and cultured on glucose asparagine agar and other media. Growth is extremely slow on all media tried. The actinomycete has been inoculated into the trunks and branches of mature grapefruit trees and typical symptoms of gummosis have resulted. These preliminary results support Godfrey's implication of an actinomycete as the cause of gummosis.

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PHYTOPATHOLOGICAL NOTES

Relation of Weather to the Severity of White Oak Anthracnose.[1] Charles L. Fergus. In a previous publication the author[2] postulated that weather conditions favoring development of the anthracnose disease of white oak caused by *Gnomonia veneta* (Sacc. and Speg.) Kleb. are: a) an open winter with temperatures of December, January and February higher than normal, b) a delayed spring with deep snow cover in March and temperatures of March and April lower than normal, c) moderate precipitation recorded for many days, and d) a reduced number of hours of sunshine (preventing rapid evaporation of moisture).

Further observations on the amount of infection on 58 white oaks in a limited area on the campus of The Pennsylvania State College have been made which, for

[1] Contribution No. 167 from the Department of Botany, Pennsylvania Agricultural Experiment Station. Authorized for publication August 14, 1952, as paper No. 1750 in the Journal Series.

[2] Fergus, C. L. 1950. An epiphytotic of white oak anthracnose in central Pennsylvania. U. S. Dept. Agr. Plant Dis. Rptr. 34: 292-294.

the most part, verify the assumption.

Disease indexes were calculated for 3 years by multiplying the number of trees in each severity class by the percentage infection: none 0, slight 10, one-fourth 25, one-third 33, one-half 50, and entire tree 100. The products were divided by 58 (total number of trees examined). Epiphytotics occurred in 1950 and 1952 (disease indexes of 37 and 43 respectively). In 1951 there was only a small amount of anthracnose (disease index of 14).

TABLE 1.—*Summary of weather conditions in State College, Pa., for 3 years as recorded by the Meteorology Dept., Penn. State College.*

Weather	63 year normal	1950	1951	1952
Average of December, January and February temperatures (°F.)	28	33	29	32
March temperature (°F.)	36	32	38	36
April temperature (°F.)	47	44	49	51
Total precipitation in April (in.)		2.0	2.9	4.7
Hours in April in which 0.01 in. or more of rain fell		70	63	101
Total precipitation in May (in.)		4.1	2.9	4.7
Hours in May in which 0.01 in. or more of rain fell		68	47	89
Percentage of hours of sunshine in April		39	46	36
Percentage of hours of sunshine in May		42	51	43
Disease index		37	14	43

Table 1 shows that the weather conditions which appear to have determined the occurrence of severe anthracnose are: a) a mild winter with temperatures higher than normal, b) moderate to high amounts of precipitation in April and May, c) many hours in April and May in which rain fell, and d) low numbers of sunshine hours in April and May. Of these 4, the rainfall seems to be the most important. Total precipitation is not so important as the number of days and hours in which rain fell.

The outbreaks in 1950 and 1952 may be explained by assuming rain to be the means of dissemination of spores of the anthracnose fungus. Edgerton[3] and Yarwood[4] have stressed the importance of this method of spread for anthracnose fungi.

The rain plays a part in dissemination and spore germination, but other factors such as relative humidity and evaporation are also important especially in germination. Table 1 shows that in the epiphytotic years the number of sunshine hours in April and May were significantly lower than in 1951. Under such cloudy conditions and with many hours of rain, moisture would not evaporate rapidly, thus allowing the maximum number of spores to germinate.

Phaenological data for the white oaks in the campus area were taken in 1952. On April 23 leaf buds had begun to swell. By April 30 they had opened and the leaves were ¼ in. long. This was probably the beginning of the critical period of infection. By May 14 the leaves were fully expanded. Table 2 illustrates the distribution of rain for the period during which the leaves could be infected.

TABLE 2. *Disease index and amount, duration and distribution of rainfall in April and May for 3 years*

Period	Hours with 0.01 in. or more rain	Days with 0.01 in. or more rain	Total rainfall in the period (in.)	Disease index for area
1950				
Last week of April	43	6	1.49	
First week of May	5	2	0.12	
Second week of May	3	2	0.06	
Third week of May	37	5	1.81	
Fourth week of May	23	4	2.11	
Totals	111	19	5.59	37
1951				
Last week of April	16	3	0.76	
First week of May	2	1	0.07	
Second week of May	15	2	0.86	
Third week of May	6	3	1.02	
Fourth week of May	24	6	0.98	
Totals	63	15	3.69	14
1952				
Last week of April	33	6	1.23	
First week of May	5	2	0.07	
Second week of May	22	5	1.49	
Third week of May	36	6	1.83	
Fourth week of May	26	4	1.69	
Totals	122	23	6.31	43

In 1950 and 1952 the total rainfall and the number of hours of rain significantly exceeded that of 1951. In addition, the hours in which rain fell were distributed over more days. The latter appears to be more important than total rainfall.

Unfortunately there is no evidence that predictions as to the severity of anthracnose in a given season may be made in early April. Thus a control program involving spraying with protectant fungicides must for the present be dependent upon the development of the weather and the leaves, similar to the insurance spraying program followed with apple scab.

Observations were also made of the incidence of white oak anthracnose in 1952 in 4 separate woodlots approximately 1 mi. from State College, Pa. A strip 70 yd. wide was chosen in each of the woodlots and every white oak was examined to determine the amount of anthracnose, until 58 trees were examined in a rectangle of 1–1½ acres. The white oaks in the woodlots and in the area on the campus were of approximately the same age, vigor and height.

The average of the disease indexes for the 4 woodlots was 20 compared to 43 for the campus area, showing that anthracnose is not so severe under natural forest conditions as it is in the park-like area examined on the campus. The explanation for this difference in severity is not known. The campus area examined is

[3] Edgerton, C. W. 1908. The physiology and development of some anthracnoses. Bot. Gaz. 45: 367-408.

[4] Yarwood, C. E. 1951. Defoliation by a rain-favored, a dew-favored, and a shade-favored disease. Phytopathology 41: 194-195.

about 1 acre in extent, and is maintained as a park area. It has been cleared of underbrush, sodded and fertilized. The fallen leaves and twigs are raked during fall and spring. The trees therefore are subject to an artificial environment, quite dissimilar to forest conditions. Although leaves or diseased leaf fragments resulting from the shattering of the brittle necrotic tissue could be missed, the value of raking and removing leaves and twigs as a method of control is doubtful.

In summary, the relation of weather to the severity of white oak anthracnose, caused by *Gnomonia veneta* (Sacc. and Speg.) Kleb., in State College, Pennsylvania, and vicinity was studied during 1950, 1951, and 1952. The factor most closely related to severity was the number of days and hours of rain during late April and May, when buds were opening and leaves expanding. Severity was greater in 1952 in a park area where leaves and twigs were raked and removed, than under natural forest conditions.—Department of Botany, The Pennsylvania State College, State College, Pennsylvania.

A Culture-Reared, Plant Parasitic Nematode Suitable For Teaching and Research. ELDON J. CAIRNS. The discovery of a *Ditylenchus* nematode responsible for serious losses in mushroom crops[1] has resulted in a finding that has other significant and broad applications. Readily culturable on fungi, there is now available a plant-parasitic nematode of the buccal stylet-bearing type which, although possibly a new species (of the genus *Ditylenchus*), is very closely related to *D. destructor*, Thorne, the potato rot nematode, and to *D. dipsaci* (Kühn), the stem or bulb nematode, both of which are obligate endoparasites of the higher plants. The ease with which great numbers of this nematode can be produced merits the attention of teachers and researchers in invertebrate zoology, phytonematology, and in testing methods aimed at nematode control.

A few general characteristics of the nematode, of value in considering its use, may be mentioned. It is of typically slender, fusiform shape. Its morphology, behavior, and entire life cycle are readily observable under the microscope (Figure 1). It also exhibits a capacity for prolonged existence in a dormant state (anabiosis) a phenomenon of biological interest and of particular importance in phytopathological considerations. Studies are in progress on this nematode's taxonomy, life history, nature of pathogenicity to fungi, and thermal-death relationships, providing an available background of information for specialists to use.

Cultures of this nematode for various research purposes have been maintained on fungi for over 3 years at the Division of Nematology. The nematode, although first discovered as a parasite feeding on mushroom hyphae, is actually not selective in requiring any certain fungus as a source of food. Nor does it require particular environmental conditions as to bacterial contamination, aeration, temperature, or light. As long as the fungus can grow, the nematode thrives. It has been reared in containers of various sizes and kinds, ranging from agar in capillary tubes containing single individuals to wooden boxes filled with mushroom-inoculated compost ultimately containing hundreds of thousands of the nematodes.

Two ways of culturing seem to meet the routine needs, either for working with relatively few nemas or for production of great numbers. For raising small populations, Petri dishes are used with potato-dextrose agar or some other more bacterial restricting medium. They are inoculated with known isolated fungi or simply exposed to the usual air-borne fungi contaminants, which have been found satisfactory. They are subsequently inoculated with the nematodes. For the production of large populations of the nemas, bottles of mushroom-spawn are inoculated with the nematodes.[2] Both ways have been used extensively in the laboratory.

Carefully made, inexpensive mushroom-spawn cultures growing in quart bottles are available from mushroom supply houses.[3] They are of 2 types. The milk bottle type is expendable and is broken to release the contents, when the nematodes have reached the desired population level. The patented, screw-top type permits removal of contents and reuse of the bottle. The spawn producers will send cultures to all parts of this country and abroad.

For bulk production of the nematodes spawn bottles have several advantages over larger or opaque containers such as boxes. Initial population build-up is more rapid in bottles and can be checked with the aid of a hand lens. A satisfactory moisture content is maintained without need of regulation. Freedom from mites and insects is maintained by the cotton stopper plus the added precaution of a paper cap. Also, there is a decided convenience in the handling and storage of the bottled cultures.

Of interest to pathologists testing methods for controlling plant parasitic nematodes is the fact that in the nema-inoculated spawn culture bottles, large quantities of the nematodes in the dormant and more resistant anabiotic state can be obtained. As the culture medium is depleted of fungi and dries out, the nematodes aggregate on the inside surface of the bottle and at the top of the culture. Thus solid masses form, made up of thousands of nematodes, while many more remain throughout the substrate also in the condition of quiescence.—Division of Nematology, U. S. Depart-

[1] Lambert, E. B., G. Steiner, and C. Drechsler. 1949. The "Cephalothecium disease" of cultivated mushrooms caused by a nematode (*Ditylenchus* sp.), evidenced by surface development of predaceous fungi. Pl. Dis. Rptr. [U. S.] 33: 252-253.

[2] This method was suggested by Dr. E. B. Lambert, Div. Fruit and Vegetable Crops and Diseases, U. S. Department Agriculture.

[3] A list of names and addresses of dealers who sell mushroom-spawn cultures will be sent upon request.

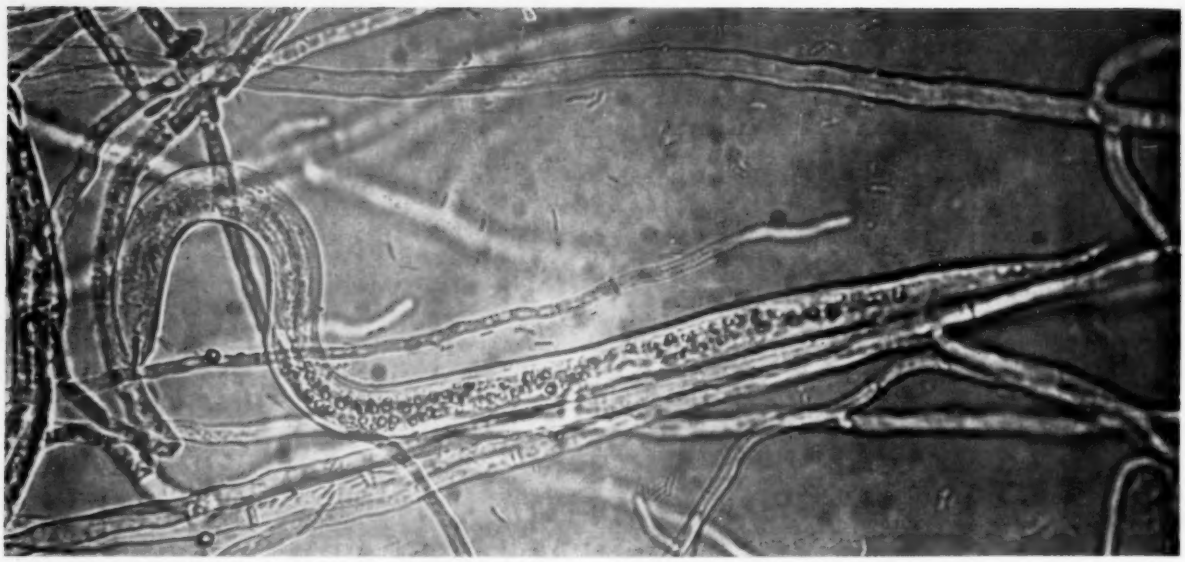

FIG. 1. *Ditylenchus* sp. nematode feeding on mushroom hypha by means of its protrusible, buccal-stylet. The hollow stylet is injected into the host cell and thru it are withdrawn the cell contents. Random, probing punctures also result in numerous minute wounds exposing the cytoplasm to bacterial action.

ment of Agriculture, Plant Industry Station, Beltsville, Maryland.

Host Preferences of the Tobacco Cyst Nematode (Heterodera sp.)[1] B. F. LOWNSBERY. In September, 1951, a *Heterodera*, morphologically resembling the golden nematode of potato, *Heterodera rostochiensis* Wollenweber, was found on a single Connecticut farm on roots of Regular Shade tobacco (3). The occurrence on tobacco suggested that there might be a difference between this nematode and the golden nematode of potato. Attempts to rear the golden nematode on tobacco in England (2, 9), Germany (8), Holland (7) and the United States (5) had been unsuccessful. The variety Regular Shade, however had not been used in these attempts.

A host preference test was run during the 1952 growing season to compare host preferences of the tobacco cyst nematode with reported host preferences of the golden nematode of potato. This test included tobacco and potato varieties and other Solanaceous species whose reaction to the golden nematode of potato is known. Six-weeks-old seedlings (or tubers of potato varieties) were planted in 3 randomized blocks in 6 in. pots of soil infested with the tobacco cyst nematode. This soil contained no other *Heterodera*. Six, 7, 8, and 11 weeks later each plant was removed from its pot and the adult *Heterodera* females on the roots around the periphery of the soil ball were counted as described by Mai (4). Results are presented in 2 groups (Table 1) since 2 lots of infested soil were used.

A rough comparison between the 2 groups is justifiable since counts for Regular Shade tobacco, included in both groups, were the same. Of the plants tested, *Solanum nigrum* L. and *Solanum burbankii* were the preferred hosts of the tobacco cyst nematode. The tobacco varieties were poorer hosts. Marglobe tomato and *Nicotiana rustica* were very poor hosts. Other species established as hosts, but not included in Table 1 because of death or poor growth of 1 or more replicates, were *Solanum integrifolium* Poir., *Solanum rostratum* Dunal. and *Solanum dulcamara* L. A few second-stage larvae but no later stages were found in roots of Katahdin and Green Mountain potato.

At least an intra-species difference between the tobacco cyst nematode and the golden nematode is shown by differences in their host preferences. Hickory Pryor, Mammoth Gold, and Gold Dollar tobacco, hosts of the tobacco cyst nematode, were among the varieties of tobacco not found to be susceptible to the golden nematode of potato on Long Island (5). The tobacco cyst nematode has not been found to mature on potato, the preferred host of the golden nematode. Larvae of the golden nematode of potato attack the preferred host of the tobacco cyst nematode, *Solanum nigrum*, but do not mature on this species (1). Dr. W. F. Mai[2] has tested *Solanum burbankii* against the golden nematode during the last 2 growing seasons, with negative results.

The morphologic resemblance of the tobacco cyst nematode to the golden nematode of potato, and the

[1] Seed for this test was kindly supplied by Drs. P. J. Anderson, E. E. Clayton, W. F. Mai and J. E. McMurtrey, and McNair Seed Company.

[2] Personal correspondence.

TABLE 1.—*Number of female tobacco cyst nematodes maturing on Solanaceous species and varieties*

Species or Variety		*No. Mature Females*[a]
Group I		
Solanum nigrum L.		283
Nicotiana tabacum L.	Havana Seed K1	68
" "	John Williams	61
" "	Regular Shade	44
" "	Havana Seed 211	41
" "	Mammoth Gold	38
" "	Turkish	30
" "	Conn. 15 Shade	29
" "	Gold Dollar	26
" "	F1 211	23
Nicotiana rustica L.		7
Lycopersicon esculentum Mill.	Marglobe	4
Solanum tuberosum L.	Katahdin	0
" "	Green Mountain	0
Solanum melongena L.	Black Beauty	0
L.S.D. at 5% level		44
L.S.D. at 1% level		60
Group II		
Solanum burbankii	Bitter[b]	210
Nicotiana tabacum L.	Burley 1 Greenville, Tenn.	84
" "	Virginia Bright Leaf	47
" "	Regular Shade	43
" "	Little Dutch	43
" "	Lizard Tail	35
" "	Kentucky 16 White Burley	28
" "	White Stem Orinoco	24
" "	Smyrna	23
" "	Hickory Pryor	22
Nicotiana alata Link & Otto (*N. affinis* Moore)		0
Nicotiana sanderae Sander		0
Nicotiana longiflora Cav.		0
Nicotiana megalosiphon Heurck & Muell Arg.		0
Solanum pseudocapsicum L.		0
Solanum tomatillo Phil. f.		0
Capsicum frutescens L.	California Wonder	0
Petunia hybrida Vilm.	Purple Prince	0
L.S.D. at 5% level		41
L.S.D. at 1% level		54

[a] Highest of 4 readings 6, 7, 8, and 11 weeks after planting in infested soil; mean of 3 replicates.
[b] Modern workers regard *Solanum burbankii* Bitter as a horticultural form of *Solanum nigrum* (C. V. Morton, Smithsonian Institution, in personal correspondence).

common hosts Marglobe tomato (5), *Solanum integrifolium* (4), *Solanum dulcamara* (6) and *Solanum rostratum* (7) possibly indicate a common origin for the 2 nematodes.

Further morphologic comparison is in progress. Unless, or until morphologic differences between the tobacco cyst nematode and the golden nematode are established, we cannot use morphology alone for their identification.—The Connecticut Agricultural Experiment Station, New Haven, Conn.

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REPORT AND ABSTRACTS OF THE 1952-53 WINTER MEETING OF THE NORTHEASTERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The annual winter meeting of the Northeastern Division of The American Phytopathological Society was held in the auditorium of the Eastern States Farmers' Exchange, Inc. at West Springfield, Massachusetts on November 6 and 7, 1952. Over 75 persons attended. States within the Northeastern Division were all represented and in addition individuals from North Carolina, Utah, and England were present.

Fifteen technical papers were presented. At the evening meeting on November 6, the guest speaker, Dr. B. M. Duggar, talked on "Sixty Years of Plant Research-Reminiscences." The morning session, November 7, concluded with a Kodachrome travelogue by Dr. D. H. Palmiter on "Impressions of the West and Some of Its Stone Fruit Virus Problems."

Officers elected for 1953 were: *President*, S. G. YOUNKIN; *Vice President*, J. S. TIDD; *Secretary-Treasurer*, A. B. BURRELL; *Councilor* for a second year, F. O. HOLMES.

J. S. TIDD,
Secretary-Treasurer

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Recent experiences with new apple fungicides.—W. A. CHANDLER, and H. W. THURSTON, JR.—Tests of fungicides in Pennsylvania in 1951 and 1952 have continued to show that several newer organic fungicides give control of apple scab equal to or better than that obtained with sulfur schedules. A distinct advantage of the newer materials is reduced injury to both foliage and fruit. In addition to recording the amount of scab and russet on fruit, the authors have used a glossmeter for measuring specular reflection from apples. This technique has resulted in quantitative measurements of one of the intangible factors entering into "finish" and has been most easily demonstrable on the Stayman variety. In 3 successive seasons it was found that the glossiest apples were those sprayed with Crag Fruit Fungicide 341, with Orthocide 406, or with combinations of these and mercury. In a study of methods of application, comparisons were made between hand spraying and mist-blower applications at full dilution and at 3 × concentration, with results indicating that several fungicides can be fully as effective and safe at this concentration as in dilute applications.

Storage behavior of Kennebec potatoes infected by Verticillium albo-atrum.—BERNARD A. FRIEDMAN and DONALD FOLSOM.—An 1800-lb. lot of Maine-grown Kennebec potatoes with approximately 97 per cent of tubers having a stem-end xylem discoloration caused by *Verticillium albo-atrum* was stored in New York City at 40° F. for 7 months. The discoloration did not change in intensity during storage or predispose the tubers to secondary infections. Waste from removal of discolored tissue was only 1.5 per cent by weight. In addition, 19.4 per cent of the tubers had a pinkish discoloration around the eyes called "pink eye," 10.6 per cent had internal brown spot, 4.3 per cent had internal cavities, not of the hollow heart type, and 2.4 per cent were decayed. Pink eye was found on many tubers from plants infected by *V. albo-atrum*, but its relation to this fungus is not known. During storage pink-eye-affected areas of the tubers became wrinkled and shriveled, and in more serious cases they were sunken, cracked, and occasionally infected by *Fusarium* and *Pseudomonas fluorescens*. Internal brown spot and cavity formation did not increase in storage. Waste from these internal defects was 5 per cent by weight. Preliminary results indicate that verticillium-wilt-infected tubers may be safely stored, but those also affected with pink eye should not be stored for a prolonged period.

Transmission of necrotic ringspot virus to a new host.—R. M. GILMER.—Japanese plum (*Prunus salicina* Lindl. vars. Abundance and Burbank) has been reported by Moore and Keitt as immune from the viruses of necrotic ringspot and cherry yellows, as evidenced by lack of symptoms following inoculation and failure to retransmit either virus from experimentally inoculated plants of either variety. Twenty varieties of *P. salicina*, including Abundance, Burbank, and several *salicina* hybrids, were propagated on peach. Inoculations of the peach understock were made from sources of sour cherry infected with 1) necrotic ringspot and 2) necrotic ringspot and yellows. Shock symptoms of necrotic ringspot appeared within 10–14 days on the *salicina* scions in both inoculation series. Symptoms in the 2 series were essentially identical and included mottle, necrosis, and shotholing. Symptoms generally were limited to 1–3 leaves only. Subsequent growth failed to exhibit symptoms. Retransmission of the necrotic ringspot virus to peach from both series was successful in several instances. The experiment was repeated on myrobalan understocks employing strains of the viruses indigenous to New York and Wisconsin. Results are in essential agreement with those obtained on peach understocks, affording evidence that immunity from necrotic ringspot virus is absent in *P. salicina*.

Further observations on antibiotic production by penicillium patulum in soil and on non-sterile manures.—ERNA GROSSBARD.—Previous studies demonstrating antibiotic production in heat treated soil by *P. patulum*, *Aspergillus clavatus* and other fungi were extended to include 5 different soils (pH 5.3–7.8). These were amended with 2.5 per cent glucose or sugar beet pulp or with 2.5–5 per cent wheat straw and inoculated with *P. patulum*. Antibiotic production took place in all soils containing glucose or sugar beet pulp but with variable yields. Differences in yield were more marked in the straw treatment and with 1 soil negative results were obtained. Apparently if an easily available source of carbon is present antibiotic production is supported by a variety of soils. A clear correlation between soil reaction and activity was not observed but the antibiotic persisted longer in acid than in alkaline soils. Treatment with propylene oxide did not sterilize soil (amendment: 2.5 per cent) completely. Nevertheless antibiotic production took place but activity was 74–83 per cent lower than in autoclaved soil. Nonsterile timothy grass inoculated with *P. patulum* supported antibiotic production but wheat straw failed to do so. However, on addition of glucose a high activity was obtained indicating that given adequate nutrients antibiotic production may take place even in the face of intense microbial competition. Low antibacterial activity was detected also in noninoculated timothy when incubated at 25°.

Peach canker caused by the fungus fusicoccum persicae E. & E.—E. F. GUBA.—For several years peach canker has caused havoc in 2 southeastern Massachusetts peach orchards. Only the current season's terminal growth is susceptible to spore infections. Water sprouts or suckers are most seriously attacked, and infections are most numerous at the nodes. The cankers are perennial and the branches die as they become completely girdled. Pycnidia and conidia develop throughout the season but the inoculum is most abundant early. Added damage results from the invasion of Fusicoccum cankers by Cytospora and other wound parasites. The etiology and epiphytology of the disease are similar to apple blotch (Phyllosticta solitaria E. & E.). There are marked differences in the reaction of peach varieties to infection. Wettable sulfur used in brown rot control is not toxic to Fusicoccum conidia and canker infections have increased. Ferbam, Thiuram, Phygon, and Orthocide are fungi-toxic. A protectant schedule of Ferbam followed by Thiuram or Orthocide in 1952 has given almost complete control of spore infections of the new growth. Protection with the new fungicides was supplemented with eradication of cankers and sanitation.

Red forcing tomatoes resistant and immune to Cladosporium fulvum Cke.—E. F. GUBA.—Improved Bay State derives its high resistance to the most virulent strain of Cladosporium and its immunity to others from *L. pimpinellifolium* (Jusl.) Mill. (U.S.D.A. Pl. Intr. 112, 215). The other parent is Bay State (Mass. Agr. Exp. Sta. Bul. 393, 1942). Waltham Mold-Proof Forcing derives its immunity to all forms of the pathogen from *L. peruvianum* (L.) Mill. recovered from segregates of crosses of (*L. peruvianum* × Prince Borghese) F_2 × Pan America made by Porte and Walker (Phytopathology 35: 931, 1945, Fig. 1). The hybrid was subsequently crossed twice with Improved Bay State. The fruits of both Improved Bay State and Waltham Mold-Proof Forcing run 3–5 to the pound, the latter being the larger of the two; of their resistant and immune ancestors about 450 to the pound.

Factors involved in the performance of Cycloheximide (Actidione) against Coccomyces hiemalis.—J. M. HAMILTON AND MICHAEL SZKOLNIK.—Actidione 2 p.p.m. controlled *Coccomyces hiemalis* when sprayed on cherry foliage after an infection period of 96 hours. Potted Montmorency and English Morello cherry trees were inoculated with conidia, given an infection period of 48 hours at 17°C. in a humidity chamber, and removed to the greenhouse. Sectioning of the infected leaves at the 96-hour interval showed that the fungus had penetrated to the palisade cells. Actidione inactivated sporulation in lesions. Artificial washing of the foliage indicated that Actidione gave protection equal to or better than Fermate, Menzate, Orthocide 406, and COCS. There was no evidence of movement of the material on the exterior of the leaf. Any sprayed leaf with functional stomates was found to be protected indefinitely. There was no indication of translocation from one leaf to another. Actidione required at least 1 hour to set to attain maximum protection. Apparently, it is not taken up rapidly as is phenyl mercury acetate. Actidione may affect size and set of fruit if applied within a 10-day period beginning with shuck split. Water with pH 7.8 did not interfere with its action. The effectiveness of Actidione was reduced with the addition of lime 3–100. It was compatible with methoxychlor.

Curvularia "fading-out" of turf grasses.—F. L. HOWARD AND M. E. DAVIES.—The sudden off-color appearance of putting greens and lawns from late June to late October has often been blamed on "drought," "iron deficiency," or "low fertility" rather than disease. Since 1949, the Velvet bent plots at the Rhode Island Agricultural Experiment Station at times have developed a yellow and green dappled color pattern from which *Curvularia lunata* (Wakker) Boed. has consistently been isolated. When the disease is severe and uncontrolled, the turf fades out leaving indefinite-edged patches of dead plants. Killed areas are seldom more than 2–3 in. wide. "Fading-out" made at least 3 epiphytotic appearances in 1952 in Rhode Island. Samples infected with *C. lunata* were also identified from Massachusetts, Connecticut, New York, New Jersey and Missouri. Characteristic yellow blotching was found affecting 80 per cent of Piper, 40 per cent of B-16 and to a lesser degree on other velvet bents (*Agrostis canina*), creeping bents (*A. palustris*), and annual bluegrass (*Poa annua*). Control with phenyl mercury acetate (Puraturf, PMAS @ 1 oz. 10 per cent active in 10 gal. water per 1,000 sq. ft.) was satisfactory in both 1949 and 1952 tests. An antibiotic (Actidione) and a water-soluble cadmium formulation (Cadsol) also proved effective in 1952.

Symptoms produced on tomato by potato leafroll virus.—JOHN J. NATTI.—Tomato plants (John Baer), 3–5 in. in height, inoculated with potato leafroll virus by means of aphids became severely diseased in the greenhouse. The first symptoms, dwarfing and chlorosis of the subsequently developed leaflets, appeared 14 days after inoculation. As growth continued, the severity of leaf symptoms increased progressively toward the apical portions of the plants. The severely dwarfed leaflets caused the plants to have a spindly appearance. The margins of the leaflets curled downward and were chlorotic. Leaflets on the lower portion of infected plants were not noticeably dwarfed. The margins of the leaflets commenced to roll upward about 20 days after inoculation. As the plants matured, the rolled leaflets became leathery in texture and developed interveinal chlorosis and anthocyanosis. Fruits on infected plants usually were more irregular in shape, of less depth, and fewer in number than those on healthy plants. On infected plants, adventitious root development stimulated by alpha-naphthaleneacetic acid in lanolin applied to the lower part of the stem was markedly suppressed. Necrotic areas in the primary phloem of infected plants gave a positive phloroglucinol test.

Fungicide mixtures as a possible means of improving apple scab and rust control.—D. H. PALMITER AND F. H. EMERSON.—Protective and eradicative fungicides were tested singly and in combinations to determine their effects on disease control and fruit finish. Spray applications were made at approximately weekly intervals without regard to infection periods. Under these conditions protective fungicides including Magnetic "70" sulfur at 6 lbs./100, Crag 341 (2-heptadecyl glyoxalidine acetate) at 3 pts./100 and Fermate (ferric dimethyldithiocarbamate) at 1½ lbs./100 averaged 64, 22, and 15 per cent fruit scab respectively. Only Fermate gave complete control of quince rust. Eradicant fungicides, Phygon XL (2,3-dichloro-1,4-napthoquinone) at ½ lb./100 and Coromerc (N-phenylmercuriethylenediamine) at ½ lb./100 held fruit scab to 3 and 5 per cent and quince rust to 1 and 3 per cent respectively. Combinations of Magnetic "70" with Phygon XL and combinations of Crag 341 with Coromerc failed to improve scab or rust control over straight Phygon XL or straight Coromerc treatments and tended to increase fruit russet. Combinations of Fermate with Phygon XL or Coromerc improved both scab and rust control over that obtained with single fungicide treatments. These combinations also tended to improve fruit finish. Fungicide 406 (N-trichloroethylthiotetrahydrophthalimide) showed both protective and eradicative properties and resulted in the best disease control and fruit finish.

The use of apple seedlings for greenhouse testing of fungicides.—M. C. RICHARDS, D. R. MURPHY, AND A. E. RICH.—The writers have found small apple seedlings grown in 2½ in. clay pots to be very useful in greenhouse testing of experimental fungicides for control of apple scab. The advantages over budded apple trees in large pots include: the much greater number of plants that can be produced and cared for in a given space; the ease with which they can be moved from place to place for inoculation, incubation, etc.; and the greater number and diversity of tests that can be run. Seeds are removed from cold storage McIntosh apples and kept in the refrigerator for planting during any month of the year. They are started in sand, then potted, and are ready for use after development

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of 4 or 5 true leaves. The seedlings may vary somewhat, but uniform plants can be obtained by selection. After use in several tests, the plants are cut back and reused. In using 3 plants for each test material 9 to 12 leaves will show infection of a diffused type after inoculation and incubation. An estimate of the infected area permits evaluation of the effectiveness of the fungicide. There has been a close correlation between greenhouse and field tests for both fungicidal value and phytotoxicity.

Susceptibility of lawn grasses to brown patch.—M. C. SHURTLEFF.—Susceptibility of lawn grasses to brown patch (*Rhizoctonia solani* Kuhn) was tested using 1-month old, greenhouse-grown plants. Twenty-nine isolates from a number of hosts, including 6 from turf, were used. Flats planted to 21 grass varieties were inoculated with 8-day old shake cultures grown on modified Czapek's medium, and then incubated at 75-85°F. and high humidity for 48 hours. Disease was assessed using the Horsfall-Barratt index where 0 is none and 11 is 100 per cent disease. The grasses were rated in order of decreasing susceptibility: Rhode Island Colonial bent (8.8); Highland Colonial bent (8.6); B-11 Velvet bent (8.5); Astoria Colonial bent (8.2); Seaside creeping bent, Meadow fescue (7.6); Redtop (7.4); Merion bluegrass (7.3); Canada bluegrass (7.2); Perennial ryegrass, Italian ryegrass, Alta fescue (7.1); Kentucky bluegrass (6.8); Annual bluegrass (6.6); Illahee fescue (6.1); Trinity fescue, Ladino clover (5.8); Creeping red fescue (5.7); Rough bluegrass (5.2); Thorne wheat (3.6); Penn. #603 corn (3.3). Each index figure represents at least 4 replications of the 29 isolates. Differences in degree of suspect injury were greater between isolates than within isolates on different grasses. Each isolate maintained its relative pathogenicity on all hosts tested. Susceptibility was consistent regardless of plant age.

Chromatographic separation of the 1-4 Naphthoquinones.—THOMAS SPROSTON, JR., AND E. G. BASSETT.—1,4-naphthoquinones are used commercially as fungicides in plant pathology and exist as naturally occurring compounds in many plants. Separation of some of the 1,4-naphthoquinones has been difficult because of insolubility, low concentration in plants, instability, etc. Paper chromatography offers an excellent means of separation of small quantities. Thirty-three different solvents were examined for good separation. Two solvents proved excellent: primary isoamyl alcohol + pyridine + water, pyridine + n-butanol + water. Good separation was attained by primary isoamyl alcohol + pyridine + water in the ratio of 3 : 2 : 1.5, and using this solvent, Rf values for the following compounds were as follows: 2,3-dichloro-1,4-NQ .53, 2-methoxy-3-hydroxy-1,4-N.Q. .69, 2-methoxy-1,4-N.Q. .85, 2,3-dimethoxy-1,4-N.Q. .90. Spots were developed by 5 per cent sodium hydroxide. This technic has been used to separate the naturally occurring 1,4-naphthoquinones in the plant *Impatients balsamina* L.

LISTING OF BOOKS

PHYTOPATHOLOGY will, from time to time as space becomes available, list titles of books on phytopathological subjects. A brief description of the contents of the books will be included.

SORAUER, P. 1952. **Handbuch der Pflanzenkrankheiten. Pflanzenchutz 6 (1): 1-448.** Ed. by O. Appel. Paul Parey, Berlin and Hamburg. price DM 78.

After a discussion of disease and insect losses, and cost of plant protection, the remainder of the volume deals with soil management and hygiene, soil treatments and disinfectants, seed storage and selection, seed treatments, and quarantines.

BAXTER, D. V. 1952. **Pathology in forest practice.** Ed. 2. John Wiley & Sons, New York. 601 pp.

This book deals with the origin, nature, causes, and control of diseases in trees in the United States, Canada, and Europe. Some of the new items treated in this second edition are: use of DDT and 2, 4-D in forest practice; prominence of virus diseases; interrelations among insects, fungi, and disease.

RAWLINS, T. E., AND W. N. TAKAHASHI. 1952. **Technics of plant histochemistry and virology.** National Press, Milbrae, California. 136 pp., price $3.50.

The greater part of the book deals with histochemical detection of compounds in plant cells and cell walls. One chapter describes some of the major technics used to study plant viruses. Short sections on elementary statistics, use of a microscope, photography, and photomicrography are included.

FREAR, D. E. H. ed. 1952. **Pesticide Handbook.** Ed. 4. College Service Publishers, State College, Pennsylvania. 176 pp., price $1.25.

Information is given on more than 4400 commercial products, indexed by trade names, active ingredients, and manufacturers.

STEVENS, N. E. AND R. B. STEVENS. 1952. **Disease in plants.** Chronica Botanica Company, Waltham, Massachusetts. 219 pp. price $4.75.

The subject matter of plant pathology is here presented in a series of 20 lectures in which attention is concentrated on general principles. After an introductory section of 2 chapters, there follow sections on causal agents, factors influencing development, and control of diseases.

WALKER, J. C. 1952. **Diseases of vegetable crops.** McGraw Hill, New York. 529 pp. price $7.50.

Information on nature and control of diseases of asparagus, bean, beet, carrot, parsnip, celery, parsley, cabbage, cauliflower, cucumber, muskmelon, watermelon, squash, pumpkin, lettuce, onion, garlic, leek, pea, pepper, eggplant, potato, rhubarb, sweet potato, spinach, and tomato is brought together. The diseases of each vegetable are discussed with regard to symptomatology, causal agents, cycle of development, and methods of control.